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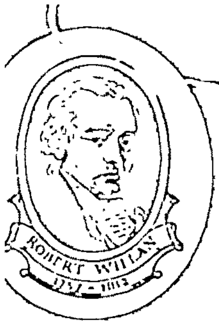

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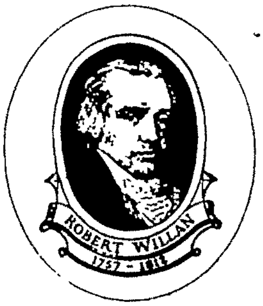

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Canad. Med. Assoc. J. 1976, **115,** 1101 J. Dermatol. Surg. Oncol 1978 **4:** 1, 91 Cutis, 1978, *21,* 491 J. Dermato Surg. Oncol. 1978, **4:** 9, 701 Canad. F h. Phys., 1976, **22,** 1301

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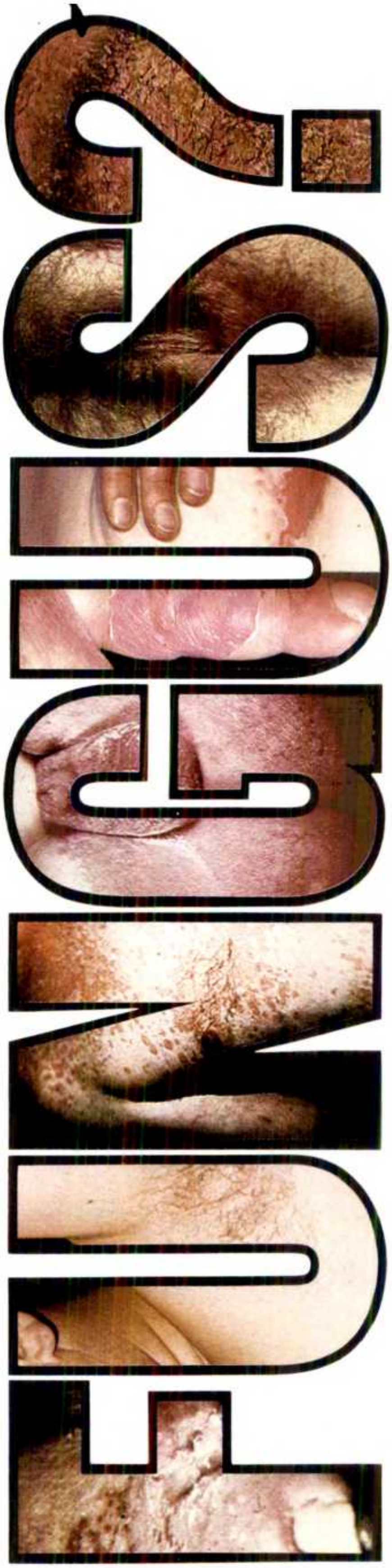

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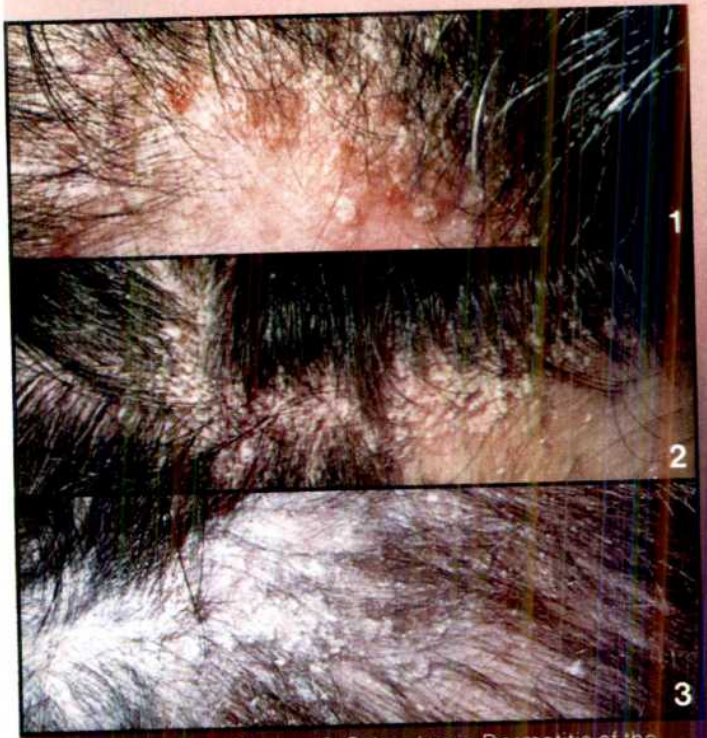

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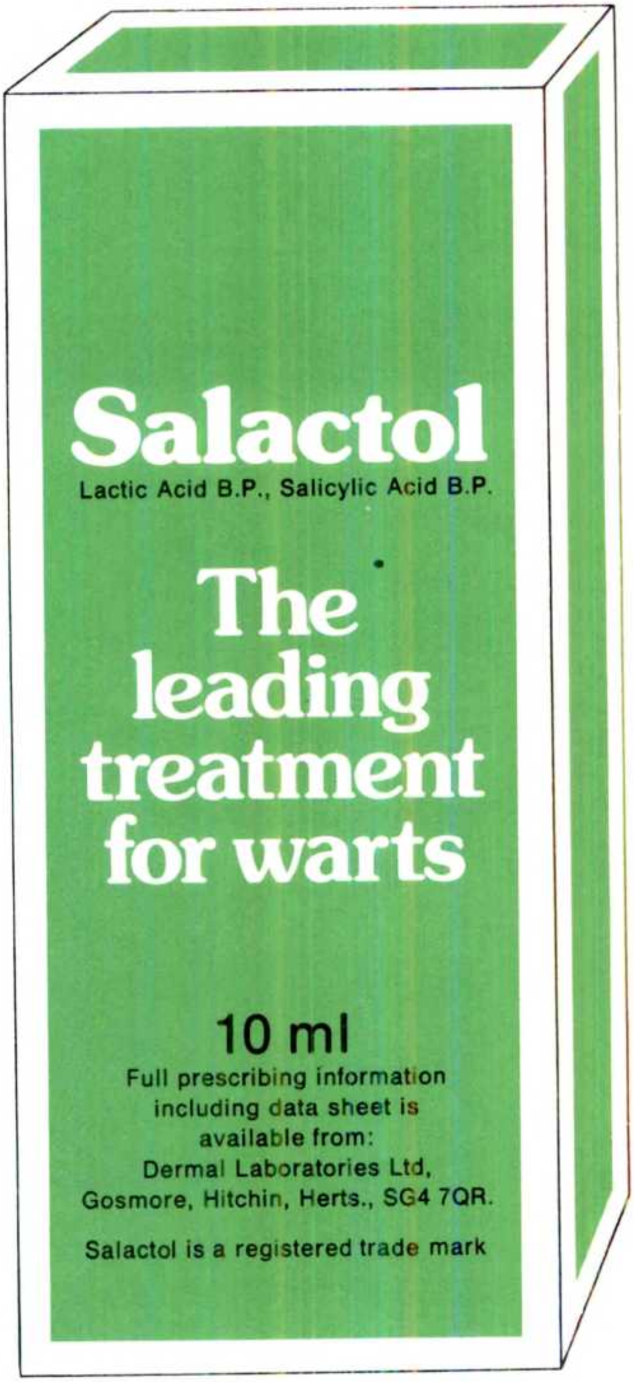

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*British Journal of Dermatology* (1980) **102**, 1.

***Clinical and Laboratory Investigations***

# Pathophysiological classification of Raynaud's phenomenon

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Accepted for publication 30 April 1979

## SUMMARY

The main types of underlying mechanism in Raynaud's phenomenon were tested separately in thirty-six patients. Within each of three groups of patients who were clinically classified according to strict definitions as having primary Raynaud's disease, systemic sclerosis or rheumatoid arthritis, there was pathophysiological heterogeneity. It is therefore suggested that in studies of treatment, at least when it is aimed primarily at one pathophysiological mechanism, the patients should be selected according to the mechanism rather than what is commonly done now, according to the associated disorder.

Studies of the different treatments of Raynaud's phenomenon (R.P.) give conflicting results (Coffman & Tudor Davies, 1975; *Lancet*, 1977). This may be due to insufficient knowledge about the underlying pathophysiological mechanisms in the individual patient and therefore the use of heterogeneous patient groups. The underlying mechanisms may be separated into exaggerated sympathetic reflexes, local mechanisms and normal microvascular reactions to cooling in patients with narrowed blood vessels and already reduced skin flow (McGrath & Penny, 1974). Each of these three groups of mechanism are thought to have differing relative importance in the pathogenesis of R.P. in the various associated disorders. In some trials the investigators have included patients with different associated disorders, and have thereby studied groups which clearly may have been pathophysiologically heterogeneous. Others have assumed that the clinical separation of R.P. into primary and secondary forms, with sub-division of those with secondary R.P. according to the type of associated disorder, produces pathophysiologically homogeneous groups (McGrath & Penny, 1974). However, it is possible that patients with the same associated disorder have R.P. caused by different mechanisms. With new techniques the main groups of mechanisms have here been assessed separately in individual patients with three different disorders, to test if the conventional clinical classification separates the patients into homogeneous groups.

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## PATIENTS AND METHODS

Thirty-six patients with R.P. (typical digital colour changes induced by cooling (McGrath & Penny, 1974) ) were studied: twelve with primary Raynaud's disease (duration of R.P. for more than 3 years without underlying disease (McGrath & Penny, 1974) ), nineteen with systemic sclerosis (P.S.S., typical skin changes confirmed by biopsy and at least clinical and radiological evidence of oesophageal involvement), and five with classical rheumatoid arthritis (R.A., A.R.A. criteria (Rodnan, McEwan & Wallace, 1977) ). The groups were matched for age, sex and duration of symptoms. Twenty age and sex matched controls with no history of abnormal cold sensitivity were also studied. Informed consent was obtained from all.

The skin blood flow of the hands was measured in a temperature controlled room at 18°C (±0·5°C) and 50% (±5%) humidity (non-sweating conditions at rest), with a previously reported Xenon-133 (Xe) clearance technique (Haavik Nilsen, 1978). Strictly standardized conditions were used throughout. The subject wore indoor clothes, had not taken vasoactive drugs for 48 h and had not smoked or consumed alcohol, coffee or tea during the 4 h before the test. For technical reasons, to avoid interference from accumulation of the Xe in the subcutaneous adipose tissue, measurements were made on the outermost 2 mm of the skin fold between the extended index finger and the abducted thumb (Haavik Nilsen, 1978).

Twenty minutes after a patient entered the room the skin fold was labelled with Xe gas by Sejrsen's (1968) non-invasive method and the clearance of radioactivity from the labelled skin fold was recorded with the patient comfortably seated. Labelling of the skin anywhere else on the hand or fingers necessitated monitoring of the clearance for at least 1·5 h to enable accurate, reproducible calculations of the skin blood flow (Haavik Nilsen, 1978). To assess the effects of cooling, the skin had to be labelled again and the clearance monitored for another 1·5 h during the application of a constant cooling stimulus. It was, therefore, not practical with this method to perform dynamic tests of the blood flow of the other areas of the skin. The clearance from the fold, following subtraction of the background radiation, was monoexponential (excluding the first 5 min after the labelling) and may be regarded as an indirect estimate of the skin fold's capillary microcirculation (Sejrsen, 1971; Haavik Nilsen, 1978). The blood flow was calculated from the conventional formula (Sejrsen, 1971):

$$\text{Flow} = (\text{In } 2/T_{\frac{1}{2}}) \times \lambda \times 100 \text{ (ml/min/100 g of tissue)}$$

where $T_{\frac{1}{2}}$ is the half-time of the recorded clearance and $\lambda$ the tissue-blood partition coefficient for the Xe (0·7 in cutaneous tissue). It was sufficient to record the clearance for 10 min only to obtain a reliable, reproducible $T_{\frac{1}{2}}$ (Haavik Nilsen, 1978).

By locally cooling a standardized area of the skin fold (2 × 0·2 cm) with a water-filled, cold (5°C) metal cylinder, skin blood flow changes were elicited which were as discussed elsewhere (Haavik Nilsen, 1978) mediated by local mechanisms and not by systemic microvascular reflexes (the local cooling test). Reflex mediated, cold-induced skin vasoconstriction was assessed by measuring the Xe clearance of the right hand for 10 min before and 10 min during standardized immersion of the contralateral (left) hand in water (the contralateral hand cooling test). At 18°C, water of room temperature was a sufficient cooling stimulus to elicit satisfactory responses, but it was too weak to produce symptomatic R.P. and was therefore not unpleasant for the patients. Tests which were repeated on the same individual were done on different days, but under identical conditions and at the same time of day. Student's '*t*'-test for non-paired variants was used (significance level: $P < 0·05$).

## RESULTS

The basal skin blood flow, that is the flow before any form of cooling, did not vary by more than 10%

TABLE 1. **Basal skin blood flow (ml/min/100 g) and the blood flow during local cooling at 5°C and contralateral hand cooling at 18°C in the four groups (mean ± s.d.)**

| | Basal flow | Local cooling | Contralateral cooling |
|---|---|---|---|
| Controls | 7·0 ± 1·4 | 7·3 ± 1·3 | 5·4 ± 1·2 |
| P.S.S. | 4·7 ± 1·5 | 2·7 ± 1·7* | 3·3 ± 1·3 |
| R.D. | 4·1 ± 1·3 | 3·8 ± 1·5 | 2·0 ± 1·1* |
| R.A. | 4·8 ± 1·2 | 4·5 ± 1·3 | 2·5 ± 1·1* |

* Decrease (absolute or %) of the basal flow significantly greater than in the controls.

when measured on the same individual on several different days, and the results of the cooling tests varied by less than 15%. All groups of patients had abnormally low basal flow at 18°C (Table 1). The change in flow during the local cooling test of the groups with Raynaud's disease (mean = 7·3% decrease) and R.A. (mean = 6·3% decrease) did not differ significantly from each other or from the normals (mean = 4·3% increase), whereas the change in flow in the group with P.S.S. (mean = 42·6% decrease) differed significantly ($P<0{\cdot}001$) from that of the normals and of the other two groups (Table 1). However, there were marked individual variations within each group and a large overlap between the groups (Fig. 1). In contrast to this, the change in basal flow during the contralateral hand cooling test of the group with P.S.S. (mean = 29·8% decrease) did not differ significantly from normal (mean = 22·9% decrease). However, the groups with Raynaud's disease (mean = 51·2% decrease) and R.A. (mean = 47·9% decrease) reacted differently ($P<0{\cdot}001$) from the normal and the P.S.S. groups, whereas there was no difference between the Raynaud's disease and R.A. groups

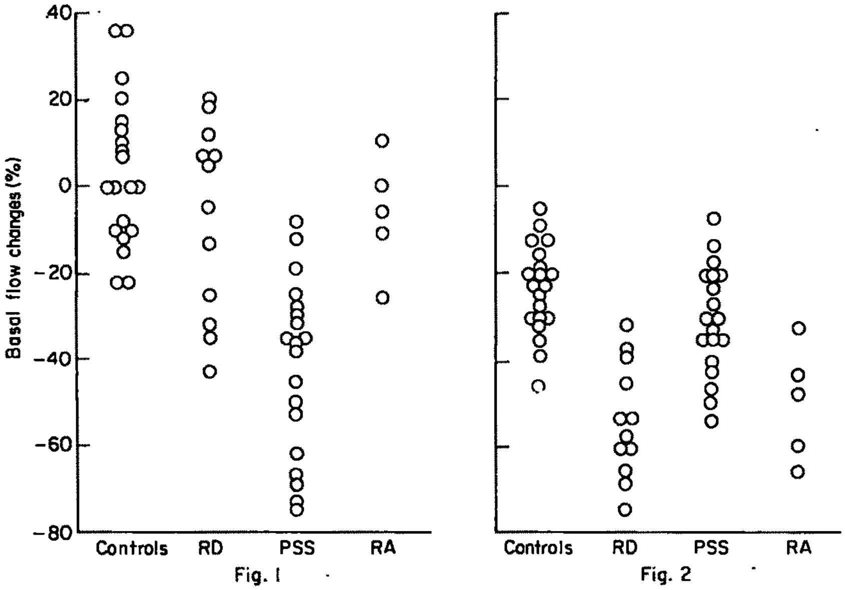

Fig. 1 Fig. 2

FIGURES 1 AND 2. **Basal skin blood flow changes (%). Fig. 1—during local skin cooling at 5°C. Fig. 2—during contralateral hand cooling at 18°C.**

themselves (Table 1). Also with this test there were marked individual variations within each group and overlap between the groups (Fig. 2). Statistical evaluation of the groups gave similar results both when the absolute change and the percent change in basal flow were used, though the overlap was more pronounced with the absolute values.

Most patients within each group had a normal response to one cooling test and an abnormal response to the other. However, some patients in each group reacted moderately abnormally to both cooling tests. In three patients (one with Raynaud's disease and two with P.S.S.) the responses to both tests were normal, but they had the lowest basal flow recordings.

## DISCUSSION

In contrast to conventional plethysmographic and thermal techniques, which give information about the total skin blood flow, the tests described here, being based on Xe clearance, measure the cold sensitivity of the capillary, nutritionally important skin circulation (Sejrsen, 1971; Haavik Nilsen, 1978). The capillary circulation may be regulated differently from the rest of the skin circulation, the main function of which is general body temperature regulation. Since the capillary flow is only a small part of the total (Coffman & Tudor Davies, 1975) any changes in it may not be detectable with the total blood flow assessment methods. This could explain why others (McGrath & Penny, 1974) estimating the total blood flow changes have failed to identify hypersensitive, cold-induced microvascular reflexes in patients with Raynaud's disease.

When the patients with, for instance, the clinically well defined entity P.S.S. were treated as a group the systemic microvascular reflexes were normal, and the local mechanisms (responses by the skin blood vessels' neuromuscular apparatus or blood constituents to localized cooling which cannot be separated by this test) were abnormal and the basal flow only moderately reduced.

However, when the patients in the group were looked at individually some were found to show the opposite, with normal responses to the local cooling test and abnormal microvascular reflexes. Some had abnormal responses to both tests and two had normal responses to both tests but markedly reduced basal blood flow. These patients did not differ from the rest of the P.S.S. patients with respect to age or duration and severity of the symptoms. This suggests that different mechanisms underlie the Raynaud's phenomenon in individual patients within this clinically apparently homogeneous group. The same pathophysiological heterogeneity was found in the Raynaud's disease and the R.A. groups. Some of the various forms of treatment are aimed at different underlying mechanisms (McGrath & Penny, 1974; Coffman & Tudor Davies, 1975; Lancet, 1977). Some of the discrepancies of earlier therapeutic trails may therefore be explained by the observed pathophysiological heterogeneity. In future therapeutic trials a more logical approach may be to study pathophysiologically homogenous groups of patients selected on the basis of the described or similar tests rather than on the basis of the conventional clinical classification.

The tests described here may safely be repeated as the local skin radiation dosage is small (less than 3 rad) and the total body radiation dosage is negligible (Haavik Nilsen, 1978).

In a double-blind, crossover study these tests helped identifying patients with R.P. who were most likely to benefit from an oral vasodilator (Haavik Nilsen, 1979), and in an open study they helped identify those who may get long-term benefit from intra-arterial injections of reserpine (Haavik Nilsen & Jayson, 1978). Using these tests it may also be possible to identify those few patients who may have a long-lasting benefit (Johnson, Summerby & Birnstingl, 1965) following sympathectomy. In patients with abnormal responses to the local cooling test other tests such as blood viscometry (Goyle & Dormandy, 1976) and assessment of fibrinolytic activity (Jarrett, Morland & Browse, 1978) may help differentiate further sub-groups of therapeutic importance.

## ACKNOWLEDGMENTS

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*British Journal of Dermatology* (1980) **102**, 7.

# Blood flow changes with naftidrofuryl in systemic sclerosis and Raynaud's phenomenon

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Accepted for publication 27 April 1979

## SUMMARY

Blood flow was measured in patients with Raynaud's phenomenon, systemic sclerosis and in normal controls by venous occlusion plethysmography on the forearm and calf, and changes in blood flow in the finger were followed by calorimetry.

Intravenous naftidrofuryl oxalate (praxilene) improved blood flow to the skin of the finger as measured calorimetrically in seven of sixteen patients with Raynaud's phenomenon and systemic sclerosis. The drug markedly reduced blood flow in the finger in all ten normal control subjects. However, naftidrofuryl increased blood flow as measured by venous occlusion plethysmography in the leg both of normal subjects and of those with Raynaud's phenomenon. In addition to these findings, no significant change in blood flow in response to the drug was observed in the forearm. The reasons for these regional differences are discussed and considered to be due to redistribution of blood flow as a result of differences in pharmacological receptor activity in different parts of the body and possibly also due to effects of central control.

Naftidrofuryl oxalate (praxilene) has been shown to increase blood flow and to be devoid of side-effects, such as hypotension and flushing. It has been used successfully in the treatment of intermittent claudication but there are no reports of its effect in systemic sclerosis and Raynaud's phenomenon. We have, therefore, studied blood flow and its response to naftidrofuryl in patients with these conditions and compared the findings with those of control subjects. The blood flow in the arm and leg was studied with venous occlusion plethysmography and the heat output from the finger was measured calorimetrically.

## PATIENTS AND METHODS

Nine patients aged between 26 and 59 with Raynaud's phenomenon, and seven aged between 63 and 68 with systemic sclerosis, were studied. Seven male and five female volunteers between the ages of 21 and 54 were controls. Naftidrofuryl, 40 mg as a 5 ml intravenous injection, was used. Five millilitres of normal saline was given as control i.v. before naftidrofuryl administration. Peripheral blood flow in the finger was measured using calorimetry (Tan, Gaylarde & Sarkany, 1978). Venous occlusion

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plethysmography using a mercury strain gauge was the method for the study of the blood flow in the calf and/or forearm. Blood flow was measured at 1 min intervals and the average of at least ten readings was used. The patients and volunteers were kept in a draught-free room at 24·5±0·5°C for at least 30 min, but longer if indicated by unstable readings, to allow the subject to become familiar with the experimental apparatus and to come into thermal equilibrium with the surroundings. The patients and subjects were not allowed to smoke for at least 4 h before the start of the study. The stirrer and chart recorder produced a continuous background noise which helped to mask external noises that might suddenly disturb the subject.

## RESULTS

The mean heat output from subjects with systemic sclerosis and Raynaud's phenomenon was less than that of the control group (Table 1). There was no correlation between heat output and age in any of these groups. Naftidrofuryl produced an increase of heat output from the middle finger in seven of sixteen patients with peripheral vascular disease and reduced it in four. All control subjects were found to have a marked reduction of heat output after intravenous naftidrofuryl. The reduction in heat output was rapid in onset and was stable at the decreased value within 2 min whereas the increase in patients with vascular disease was delayed by 10–20 min and the blood flow improved gradually. The reduction in heat output in normal subjects was apparent for at least 40 min, with little diminution in

TABLE 1. Heat output from the finger measured calorimetrically showing the effect of naftidrofuryl on the superficial blood flow of normal and diseased subjects

| Subjects | Heat output before naftidrofuryl (W) Mean | s.e. | Heat output following 40 mg naftidrofuryl as percentage of control values Geometric mean | s.e. geometric mean |
|---|---|---|---|---|
| Normal controls (N = 10) | 2·312 | 0·509 | 46·7 | 1·115 |
| Raynaud's patients (N = 9) | 0·633 | 0·100 | 110·6 | 1·216 |
| Systemic sclerosis patients (N = 7) | 0·410 | 0·105 | 138·2 | 1·264 |

TABLE 2. Effects of saline and naftidrofuryl on blood flow in the forearm and calf measured by venous occlusion plethysmography

| Subjects and treatment | Geometric mean of change in blood flow expressed as percentage of control flow Leg | s.e. | N | Arm | s.e. | N |
|---|---|---|---|---|---|---|
| Saline controls after 5 ml saline | 103·3 | 1·047 | 13 | 85·5 | 1·064 | 12 |
| Normal controls after 40 mg naftidrofuryl in 5 ml saline | 113·7 | 1·053 | 11 | 103·2 | 1·136 | 7 |
| Raynaud's patients after 40 mg naftidrofuryl in 5 ml saline | 111·8 | 1·035 | 9 | 87·3 | 1·045 | 5 |
| Systemic sclerosis patients after 40 mg naftidrofuryl in 5 ml saline | 95·7 | 1·061 | 6 | 98·2 | 1·094 | 6 |

effect at the end of this time. Venous occlusion plethysmography showed increased blood flow following naftidrofuryl in legs of normal controls and in subjects with Raynaud's phenomenon and decreased flow in the legs of patients with systemic sclerosis. No significant change of blood flow occurred in the forearm in response to naftidrofuryl (Table 2). In contrast, intravenous saline reduced the blood flow in the forearm and had no effect on the leg.

## DISCUSSION

These results show that the effect of naftidrofuryl on blood flow is complex. Since naftidrofuryl does not alter blood pressure and heart rate (Fontaine *et al.*, 1969), the reduction in skin flow in normal subjects is the result of either a fall in cardiac output or vasodilatation elsewhere. The injection of normal saline caused only a transient drop in heat output from the finger, lasting about 2 min, but some workers have noted a more long lasting effect on skin flow in man. We also found that the blood flow in the arm is reduced for at least 10 min after saline injection in the contralateral arm.

Blood flow in the arm varies rapidly with time. This variation is a result of normal homeostasis, external disturbance and the trauma of injection. The flow in the leg is more constant and Table 2 shows that the standard error of the results is not as great as in the arm. The increase in blood flow in the leg in response to naftidrofuryl is consistent with reports from clinical trials that the drug is of use in the treatment of intermittent claudication (Kappert, 1967; Sicard *et al.*, 1968; Czepak, 1973), although Ruckley *et al.* (1978) reported no improvement in their series.

Naftidrofuryl caused vasoconstriction in the fingers while producing an increased blood flow in the leg of normal subjects. This is not as unexpected as it might seem at first, since even the powerful vasodilator amyl nitrite causes an intense vasoconstriction in the fingers (Burton, 1961). The latter was considered to be due to centrally controlled mechanisms.

We have also recently found that ethanol causes different regional changes in skin blood flow and similar observations were previously made by Fewings *et al.* (1966) using hand and limb plethysmography. These results show that the effect of vasodilators is not uniform in all areas of the body and that the response of the hands in particular may be different from other parts. It is well known, for example, that blood vessels in the fingers constrict in response to adrenaline, where the blood flow in the forearm initially increases and subsequently returns to preinfusion values (Whelan, 1954). Other factors causing differential flow in the hand and forearm include nicotine, painful stimuli, $CO_2$ inhalation, hyperventilation and acetyl choline.

All these observations suggest that the use of vasodilators in Raynaud's phenomenon and systemic sclerosis has to be reappraised and based on both clinical response and objective measurement of blood flow. We agree with Coffman (1979) that an ideal drug for the treatment of peripheral vascular diseases would dilate blood vessels and increase blood flow only in areas of deficient blood supply but, unfortunately, these agents do not yet exist. Fortunately, however, in vasospastic diseases, vasodilator drugs may have a beneficial effect in many patients by increasing cutaneous capillary blood flow through their action on the sympathetic nervous system.

We have shown that naftidrofuryl in some patients with systemic sclerosis and Raynaud's phenomenon produces an increase in blood flow and it would seem that this is due to its selective effect on abnormal blood vessels. We know of no other report which has suggested that diseased blood vessels respond to vasodilators in the opposite way to that seen in control subjects.

## ACKNOWLEDGMENTS

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# The *in vitro* effect of a calf thymus extract on the peripheral blood lymphocytes of patients with warts

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## SUMMARY

T and B lymphocyte populations were evaluated in forty-one patients with multiple recurring warts. Active rosettes (T-Ea) and total T lymphocytes (T-Et) were significantly decreased.

The *in vitro* effect of a calf thymus extract (TP-1) on peripheral blood lymphocytes was evaluated in thirty-three patients. A statistically significant increase in E-rosette forming cells was observed in twenty-three patients (69%). Serum blocking factors were not found in the patients with a positive response to TP-1, which suggests the possibility of a thymic hormone deficiency. In the case of non-responders to TP-1, on the other hand, an anti-lymphocyte antibody capable of directly blocking the hormone receptors on the undifferentiated lymphocytes may be postulated.

Cell-mediated immunity is known to play the most important part in the control of viral infections. Recurring disseminated warts in patients with T lymphocyte depression are a frequent clinical finding (Spencer & Andersen, 1970), while the incidence of warts increases to a considerable extent after treatment with immunosuppressive drugs. A patient with a long history of warts studied by Morrison (1974) displayed depressed cell-mediated immunity in the leukocyte migration test, coupled with increased immune response during periods of resolution. A reduced reactivity to tuberculin by comparison with healthy controls was reported in children with warts by Brodersen, Genner & Brodthagen (1974).

Rosette formation with sheep erythrocytes has been used both to count the total circulating T-lymphocyte population (T-Et) and to measure active T-lymphocytes (T-Ea). This test may be influenced by many pathological conditions, such as virus diseases. Recent work has emphasized the predominant part played by the thymus hormones in the maturation and differentiation of T-lymphocytes. Thymus extracts, for example, will induce lymphoid cells to acquire distinctive T-cell surface markers (Bach, Dardenne & Goldstein, 1971; Komuro & Boyse, 1973). Incubation of peripheral blood lymphocytes with thymus hormone results in an increased number of E-rosette forming cells (E-RFC)

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in patients with primary immunodeficiency diseases (Wara *et al.*, 1975; Goldstein *et al.*, 1976), cancer (Goldstein *et al.*, 1976; Bernengo *et al.*, 1979), systemic lupus erythematosus (Scheinberg, Cathcart & Goldstein, 1975) and virus infections (Wara *et al.*, 1975; Goldstein *et al.*, 1975).

In this paper, we report the T and B cell pattern in forty-one patients with disseminated warts that had recurred for periods of more than 1 year. The *in vitro* effect of a calf thymus extract on their peripheral T cells was also investigated.

## MATERIALS AND METHODS

Seventeen males and twenty-four females aged 16–50 years with a long history of numerous warts (refractory to conventional treatment) on the hands (twenty-four cases), feet (six cases), hands and feet (two cases) and face (nine cases), unaccompanied by concomitant diseases, were studied.

Freshly drawn blood was mixed with heparin (20 u/ml) and plasma gel (4 vol. of blood, 1 vol. of plasma gel) and allowed to sediment at 37°C for 1 h. The supernatant was layered onto 4·0 ml of Lymphoprep gradient. After centrifugation at 400 *g* for 30 min at room temperature, the mononuclear cells at the interface were removed by aspiration, washed three times with Hanks's balanced salt solution (HBSS) and resuspended in McCoy's without fetal calf serum (F.C.S.). Cell viability (95–98%) was determined by the trypan blue exclusion test. Sheep red blood cells (SRBC) were stored at 4°C in Alsever's solution. Before use the cells were washed twice and adjusted to a 0·5% suspension.

### *E rosettes*

E and EAC rosettes were determined according to the WHO/IARC Workshops' Special Technical Report (1974) with a few modifications. Total T lymphocytes (T-Et): 0·25 ml of the lymphocyte suspension was mixed with 0·25 ml of 0·5% SRBC (SRBC/lymphocyte ratio 30:1) and incubated at 37°C for 5 min. The suspension was spun at 200 *g* for 5 min and then incubated in ice for 18 h. Then 0·25 ml of the supernatant was removed, the pellet was gently resuspended by shaking and at least 300 lymphocytes were counted under a light microscope at a magnification × 1000. Lymphocytes binding three or more SRBC were considered positive. T-Ea were determined in the same way as T-Et using lymphocytes resuspended in McCoy's containing 10% heat inactivated FCS previously absorbed with sheep red blood cells. After incubation at 37°C for 5 min and after centrifugation the cells were immediately resuspended and counted.

### *Incubation with the thymus extract*

The calf thymus extract (TP-1 kindly provided by the Istituto Farmacologico Serono Roma) was prepared according to the method previously described by Falchetti (Falchetti, Bergesi & Caprino, 1976; Falchetti *et al.*, 1977; Bergesi & Falchetti, 1977). Lymphocytes were resuspended in McCoy's without FCS; 0·25 ml of the lymphocytes suspension was mixed with 0·25 ml of 0·5% SRBC and 250 μg/ml of TP-1 and incubated at 37°C for 5 min. The suspension was spun at 200 *g* for 5 min and then incubated at 4°C for 18 h. Then 0·25 ml of the supernatant was removed, the pellet was resuspended and rosettes counted. Different concentrations of TP-1 were used in our experiments to determine the optimal dose (50, 100, 200, 250, 300 and 500 μg/ml). In normal and in wart patients, lymphocyte populations had the same optimum TP-1 concentration. Control studies were carried out with spleen extract prepared in the same way as TP-1.

Surface immunoglobulins were determined in accordance with Pernis, Forni & Amante (1970) using monovalent fluorescein-conjugated goat anti human immunoglobulins sera (anti IgG, IgA, IgM, IgE, IgD).

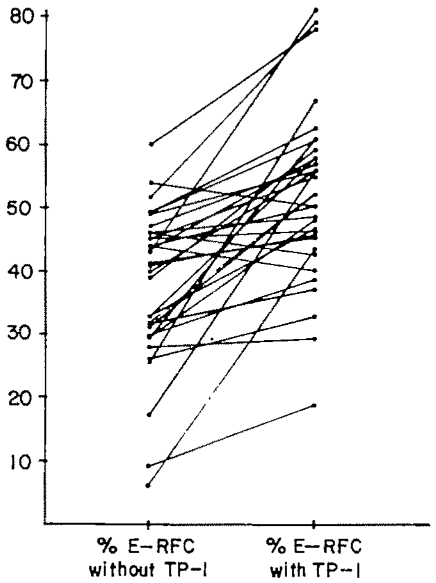

FIGURE 1. *In vitro* effect of TP-1 on E-RFC in patients with warts.

### *Rosette inhibition studies*

Whole serum from eight patients with warts and the supernatant from an overnight culture of lymphocytes of two patients were studied for ability to inhibit E-rosette forming cells (E-RFC) by normal lymphocytes from six healthy controls. Baseline E-rosette assays were performed with and without TP-1. Normal lymphocytes (0·25 ml) were incubated for 30 min with 0·03 ml of heat decomplemented serum from wart patients and with 0·03 ml of the supernatant from overnight cultures. E-RFC tests were performed with and without TP-1.

## RESULTS

T-Ea, T-Et and null cell values in the forty-one patients and twenty-four normal subjects are shown in Table 1. A significant ($P < 0·01$) reduction in per cent and absolute T-Ea and T-Et values was noted, whereas EAC levels and the percentage of lymphocytes with surface immunoglobulins (SmIg) (Table 2) displayed no significant changes with respect to the controls. A significant increase in absolute and per cent null cell values was also apparent. It may be pointed out that T-Ea, T-Et and

TABLE 1. T and B cells in peripheral blood of forty-one patients with warts

| | % T-$E_a$ | % T-$E_t$ | % EAC | % Null cells | Absolute lymphocyte count/$mm^3$ |
|---|---|---|---|---|---|
| Warts (41) | 20·7±12·6 | 41·9±17·1 | 17 ±7·2 | 39·4±18·1 | 2580±1184 |
| Controls (24) | 31·9± 8·9 | 64·7± 7·6 | 17·5±9·3 | 17·6±10·5 | 2425± 706 |
| Student's '*t*'-test | $P < 0·05$ | $P < 0·0001$ | N.S. | $P < 0·0001$ | N.S. |

TABLE 2. SmIg positive cells in peripheral blood of patients with warts

| | % SmIg | | | | |
|---|---|---|---|---|---|
| | IgG | IgA | IgM | IgE | IgD |
| Warts | 5·8±3·7 | 2·7±2·6 | 5·6±2·6 | 0·7±0·7 | 3·6±2·8 |
| Controls | 6·4±3·4 | 3·0±1·6 | 6·8±1·9 | 0·8±0·8 | 4·3±1·5 |

null cell values in patients with few, readily treatable warts were similar to those observed in the controls. Incubation with TP-1 led to an overall increase in the number of E-RFC from 37·6±12·3 to 51·8±13·5 ($P<0·01$) in a group of thirty-three patients. TP-1 was effective in twenty-three of thirty-three patients (69%) and in twelve of these E-RFC returned to normal (Fig. 1). No such increase was noted in the controls: 64·7±7·6 vs 62·5±6·8.

Serum from eight of these patients with low baseline E-RFC was incubated with lymphocytes from six normal subjects. No change in E-rosette forming capacity was induced with the sera from the six patients with a positive response to TP-1. Sera from two patients who did not respond, however, led to decreases from 50% to 27% and from 52% to 17% respectively. Simultaneous incubation with TP-1 gave only partial protection, 36% and 34%. Incubation of normal lymphocytes with the supernatant obtained after overnight culture of lymphocytes from these eight patients was also investigated. No change in rosette numbers was produced by the supernatant from the six responders, whereas that from the two non-responders led to a fall from 53% to 18·5% and 28% respectively; in this case, simultaneous incubation with TP-1 prevented a fall in E-RFC numbers.

## DISCUSSION

T-lymphocyte depression was evident in this series of patients with multiple refractory warts, as reported by other workers (Chretien, Esswein & Garagusi, 1978). This depression affected both the total T population and the active rosette sub-population, which is regarded as the sub-population most actively engaged in cellular immunity (Fudenberg, Wybran & Robbins, 1975).

A blocking substance on the T-lymphocyte surface would offer one way of explaining this depression. Moroz *et al.* (1977) found ferritin on the surface of lymphocytes from patients with Hodgkin's disease and suggested that it was removed by levamisole, while Michalevicz *et al.* (1978) were of the opinion that the increase in E rosettes noted after incubation of T lymphocytes from patients with lupus erythematosus in the presence of levamisole and thymic humoral factor can be attributed to the removal of a blocking substance from their surface. Moutsopoulos *et al.* (1976) have shown that the depressed percentage of E-RFC in disseminated lupus is probably due to cell-bound lymphocyte antibodies that block the T-cell receptors for sheep erythrocytes, and possibly those for thymosine on the undifferentiated lymphocytes. Diminished T-lymphocyte differentiation may provide another explanation of depression. Our patients, in fact, displayed normal B-lymphocyte levels and an increased null cell population. In addition to stem cells, this population includes post-thymic ($T_0$) cells, which do not form E rosettes, are insensitive to anti-T sera, and lack the functional characteristics of the T lymphocytes. When incubated with thymus hormone, however, they become capable of forming E rosettes. The *in vitro* response to TP-1 noted in our patients suggests that most of their null cells were $T_0$ cells.

The presence of serum blocking factors in these patients cannot be totally excluded by the observation that incubation with either their sera or the supernatant of overnight culture of their lymphocytes did not decrease the number of E-RFC in normal subjects.

In the case of the non-responders to TP-1, on the other hand, an anti-lymphocyte antibody capable of direct block of the hormone receptors on the undifferentiated lymphocytes may be postulated, since their heat-inactivated sera and the supernatant of overnight culture of their lymphocytes depressed rosette formation of normal lymphocytes. It may be supposed that the blocking factor or factors were detached from the lymphocyte surface and distributed into the supernatant during overnight culture, seeing that these lymphocytes proved responsive to TP-1 when washed after such culture. In addition, normal lymphocytes were protected against inhibition by the supernatant when incubated in the presence of TP-1, probably because the red cell receptor sites were kept apart from the antigen sites for the lymphocytotoxic serum, due to the changes induced by the hormone.

In immunodeficiency, T-lymphocyte depression is often the result of faulty differentiation caused by a lack of circulating thymus hormone. In patients with warts it is possible that temporary low levels of circulating thymic hormone may occur. Spontaneous regression in these patients may thus be attributable to correction of this hormone deficiency, and hence the recovery of cell-mediated immunity.

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# Lysosome destruction and lipoperoxide formation due to active oxygen generated from haematoporphyrin and UV irradiation

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## SUMMARY

The lysosomal enzymes, acid-phosphatase and β-glucuronidase, were released from rat liver lysosome when exposed to 400 nm irradiation in the presence of haematoporphyrin, and the release was prevented by adding vitamin E, diazabicyclo-octane, bovine serum albumin, superoxide dismutase or D-mannitol to the reaction mixture. Monochromatic irradiation with wavelengths from 380 to 410 nm caused no significant differences in the release of lysosomal enzymes, but 420 nm irradiation caused three-fifths of that of 400 nm irradiation. The malondialdehyde level in rat liver homogenate increased after 400 nm irradiation in the presence of haematoporphyrin. Reduction of nitroblue-tetrazolium was not observed when haematoporphyrin was excited by 400 nm; it was considered that superoxide anion radical ($O_2^-$) was not primarily generated.

The following mechanism was assumed: that porphyrin which had been excited by 400 nm, converted ground-state molecular oxygen ($^3O_2$) to excited singlet oxygen ($^1O_2$), which formed lipid peroxides in lysosomal membrane resulting in destruction of the membrane; skin changes would occur from these released lysosomal enzymes.

The mechanism of skin photosensitivity in porphyria is unclear. Allison, Magnus & Young (1966) suggested that the photosensitivity was partially due to damage of lysosomes in the skin by free radicals which were generated from porphyrin and light. In the literature, the effect of UV with shorter wavelengths, UV-B or UV-C, was reported to cause damage to lysosomes (Slater & Riley, 1966; Johnson & Daniels, 1969; Ogura & Knox, 1973). On the other hand, Allison *et al.* (1966) showed photodynamic damage to cultured cells using 400 nm light in the presence of uroporphyrin I, although the cell cultures were not lysosome preparations.

The present paper is aimed to investigate: (1) the influence of monochromatic 400 nm light on rat liver lysosomes in the presence of haematoporphyrin; (2) the effect of scavengers for active oxygen on the lysosomal releases; and (3) the lipoperoxide formation in rat liver homogenates due to haematoporphyrin and 400 nm.

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## MATERIALS AND METHODS

### *Preparation of lysosomes*

The liver from each of twelve female albino rats weighing about 200 g was obtained immediately after decapitation, and the lysosomes were prepared from 4·0 g liver by the method of Kato (1972) and suspended in 23·0 ml of 0·25 M sucrose solution (Fig. 1).

### *Light source*

Grating monochromator with a xenon lamp (2 kW, SS-25NX type, Japan Spectroscopic Co. Ltd) was used in the experiments. The apparatus emitted a narrow wavelength of UV rays in half-band width of 5 nm.

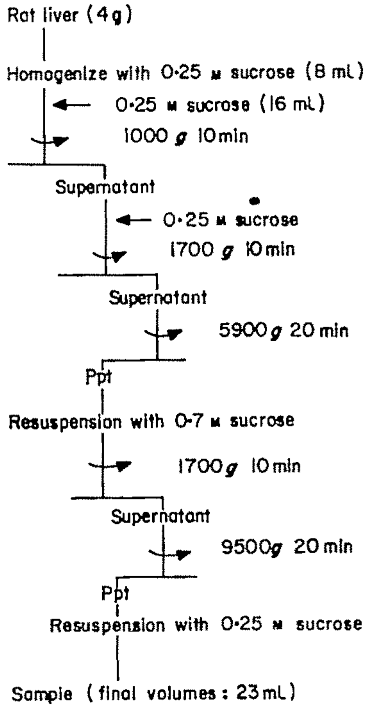

FIGURE 1. Preparation of rat liver lysosome.

### *Chemicals*

Nitroblue-tetrazolium (NBT), bovine serum albumin (BSA), D-mannitol and 1,4-diazabicyclo-2,2,2-octane (DABCO) were purchased from Wako Pure Chemical Co., Japan. Haematoporphyrin dihydrochloride (HP) was obtained from Nakarai Chemical Ltd, Japan and superoxide dismutase (SO-D) from Sigma Chemical Co., U.S.A. Na vitamin E phosphate (VE) was supplied by Eisai Co. Ltd, Japan.

### *UV and visible irradiation*

(*a*) *Reduction of NBT.* Two and a half millilitres of physiological saline and 0·5 ml of $7{\cdot}4 \times 10^{-4}$ M HP were pipetted into a specially made quartz cell (4 × 2 × 1 cm, Nogawa Glass Ltd, Japan), and 1·0 ml of NBT (1·0 mM or 0·1 mM) was added to the cell to make up a total volume of 4·0 ml. The mixture was irradiated at 400 nm with the monochromator at room temperature. Immediately after the irradiation, absorbancy of the solution was read at 560 nm using a Hitachi UV-VIS spectrophotometer.

(*b*) *Irradiation with different wavelengths.* One millilitre of $7{\cdot}4 \times 10^{-4}$ M HP was added to six cells each containing 3·0 ml of lysosomal suspensions as prepared above. Five cells were irradiated with UV or visible rays at 0°C and one was kept for control. Lysosomal enzyme activity ($\beta$-glucuronidase) was determined, immediately after the irradiation, in the supernatant obtained by centrifugation at 20,000 *g* for 30 min.

(*c*) 400 *nm irradiation of lysosomal preparations.* A mixture of 3·0 ml of lysosomal fractions and 1·0 ml of various amounts of HP ($7{\cdot}4 \times 10^{-4}$ M, $2{\cdot}5 \times 10^{-4}$ M and nil) were respectively irradiated by 400 nm at 0°C, and lysosomal enzyme activities (acid-phosphatase and $\beta$-glucuronidase) were determined in the supernatant obtained after 20,000 *g* centrifugation for 30 min. Recovery rate of the procedure was calculated from the values obtained before and aftei the centrifugation.

(*d*) *Effect of scavengers for active oxygen.* Two and a half millilitres of lysosomal fractions, 1·0 ml of $7{\cdot}4 \times 10^{-4}$ M HP, 0·5 ml of VE (antioxidant) (100 $\mu$M, 10 $\mu$M, 0 $\mu$M) in physiological saline, were mixed in the quartz cell and irradiated by 400 nm at 0°C. $\beta$-Glucuronidase activity in the supernatant was measured after centrifugation at 20,000 *g* for 30 min. A similar determination was made using DABCO as scavenger for singlet oxygen, BSA as eliminator for lipoperoxide, D-mannitol as scavenger for hydroxyl radical and SO-D as catalytic scavenger for superoxide anion radical instead of VE.

(*e*) *Lipoperoxide formation in rat liver homogenate.* One gram of liver, washed with phosphate buffer (0·05 M, pH 7·4), was dispersed in 5·0 ml of the same buffer with a teflon-glass homogenizer. The homogenate was then diluted twenty-four times with buffer and 3·0 ml of the diluted homogenate was mixed with 1·0 ml of buffered HP ($7{\cdot}4 \times 10^{-4}$ M, pH 7·4), or with buffer without HP, and irradiated with 400 nm in a quartz cell at room temperature. Lipoperoxide in the irradiated solution was assayed immediately after exposure (Masugi & Nakamura, 1977) (Fig. 2). The standard calibration curve of malondialdehyde (MDA) was made using 1,1,3,3-tetraethoxypropane, which was dissolved in a 0·1 N HCl-ethanol solution, instead of 0·5 ml homogenate in Fig. 2.

### *Enzyme and protein assay*

Acid phosphatase activity was estimated by the method described by Shibko & Tappel (1965). The enzyme preparation (0·5 ml) was mixed with 0·5 ml of 0·02 M p-nitrophenyl phosphate and 1·0 ml of 0·2 M acetate buffer (pH 5·0), and incubated for 30 min at 37°C. After the incubation, 0·1 ml of the reaction mixture was transferred into another test tube containing 4·9 ml of 0·1 M $Na_2CO_3$. Nitrophenol liberated was determined from the intensity of absorption at 400 nm. $\beta$-Glucuronidase activity was determined by the method of Talalay, Fishman & Huggins (1946). 0·2 ml of the enzyme preparation, 0·7 ml of 0·1 M acetate buffer (pH 4·5) and 0·1 ml of 1·0 mM phenolphthalein-$\beta$-glucuronide were mixed in a test tube and incubated for 30 min at 37°C, and the reaction was stopped by adding 3·0 ml of 0·4 M glycine buffer (pH 10·5); the phenolphthalein liberated was estimated by reading the intensity of absorption at 552 nm. Protein determination was performed by the procedure of Lowry *et al.* (1951).

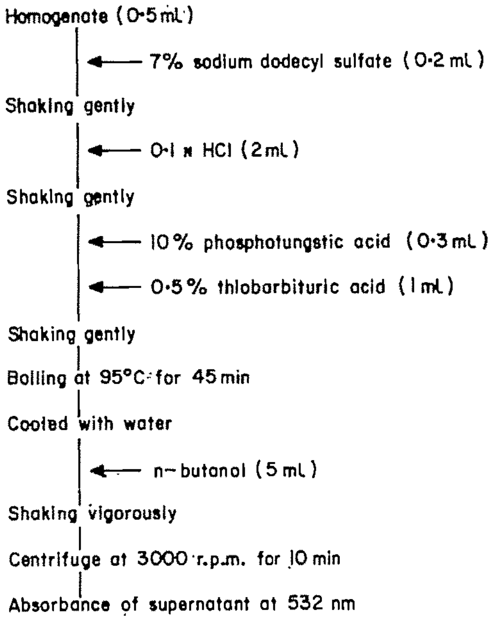
Homogenate (0·5 mL)
7% sodium dodecyl sulfate (0·2 mL)
Shaking gently
0·1 N HCl (2 mL)
Shaking gently
10% phosphotungstic acid (0·3 mL)
0·5% thiobarbituric acid (1 mL)
Shaking gently
Boiling at 95°C for 45 min
Cooled with water
n-butanol (5 mL)
Shaking vigorously
Centrifuge at 3000 r.p.m. for 10 min
Absorbance of supernatant at 532 nm

FIGURE 2. Procedure of measurement of malondialdehyde in rat liver homogenate.

## RESULTS

### *Reduction of NBT*

Reading of the absorbancy was performed at 560 nm on NBT solution to ascertain whether or not the superoxide anion radical ($O_2^-$) was generated when HP was excited by 400 nm. Irradiation energies given were 0·2, 0·4, 0·8 or 1·0 J/4 ml, respectively. The results showed that there was no change in the absorbancy at 560 nm between irradiated and non-irradiated system in both reaction systems in which the concentrations of NBT were 250 and 25 $\mu$M. It was confirmed, therefore, that $O_2^-$ was not primarily generated in this system because no reduction of NBT was observed.

### *Release of lysosomal enzyme when irradiated with different wavelengths*

$\beta$-Glucuronidase activity was measured as a marker of released enzymes. The reaction was carried out under the following conditions: the irradiation energy was 1·5 J/4 ml and the final concentration of HP was $1{\cdot}9 \times 10^{-4}$ M. Irradiation was performed at various wavelengths as shown in Table 1. The activity was expressed as $\mu$g of phenolphthalein liberated after 30 min incubation per mg protein. The activity of the non-irradiated control was 133±10 (mean±s.d.) $\mu$g/mg protein/30 min. On the other hand, there was no significant difference in activity at 380, 390, 400 and 410 nm ($P > 0{\cdot}1$), but the sample irradiated with 420 nm showed three-fifths of the activity of that irradiated with 400 nm ($P < 0{\cdot}01$).

### *Release of lysosomal enzymes by 400 nm irradiation*

Acid phosphatase and $\beta$-glucuronidase activity in the supernatant obtained by centrifugation at 20,000 *g* for 30 min were estimated after being mixed with HP and irradiated at 400 nm. The dose-response curves obtained are summarized in Fig. 3. Recovery rates during the centrifugation were acid-phosphatase 112·7±11·2% (mean±s.d.), $\beta$-glucuronidase 99·1±11·5%. Increases in activity

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TABLE 1. β-glucuronidase activity released in the supernatant after irradiation with different wavelengths. Irradiation energy = 1·5 J/4 ml; HP concentration = $1{\cdot}9\times10^{-4}$ M, n = 3

| Irradiated wavelength (nm) | Activity (phenolphthalein μg/mg protein/30 min) (mean ± s.d.) | Student's '*t*'-test (compared with the value at 400 nm) |
|---|---|---|
| Non-irradiation | 133 ± 10 | $0{\cdot}001 > P$ |
| 380 | 398 ± 42 | $0{\cdot}3 > P > 0{\cdot}2$ |
| 390 | 410 ± 29 | $0{\cdot}4 > P > 0{\cdot}3$ |
| 400 | 441 ± 36 | — |
| 410 | 398 ± 25 | $0{\cdot}2 > P > 0{\cdot}1$ |
| 420 | 256 ± 36 | $0{\cdot}01 > P > 0{\cdot}001$ |

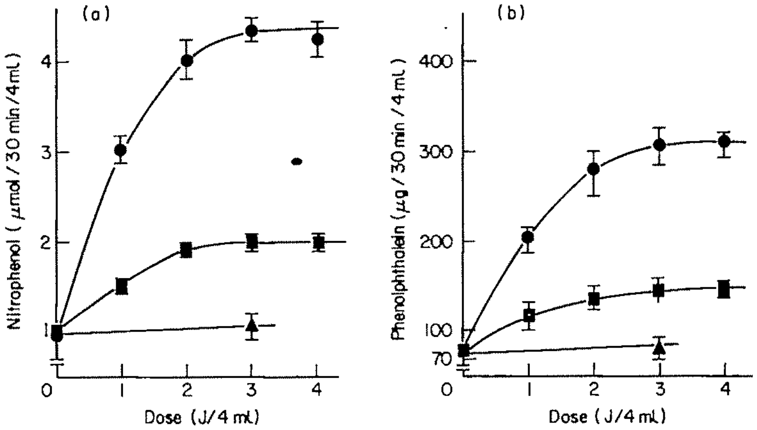

FIGURE 3. Activities of lysosomal enzymes released into the supernatant after exposure to 400 nm. (a) Acid-phosphatase; (b) β-glucuronidase. n = 5; (●) HP = $1{\cdot}9\times10^{-4}$ M; (■) HP = $6{\cdot}2\times10^{-5}$ M; (▲) HP = 0 M.

in the supernatant were proportional to the amounts of energy given, but they reached a plateau after 2 or 3 J/4 ml. As shown in Table 2, the effects of HP and 400 nm irradiation on enzyme release from lysosomes were clearly shown. With the same irradiation energy, the more HP in solution, the more enzymes released. On the other hand, activities of the two enzymes showed no significant difference when irradiated with 400 nm without HP, and/or with HP without irradiation.

### *Effect of various scavengers on enzyme release from lysosomal fraction following 400 nm exposure in the presence of HP*

The results are summarized in Figs 4–7. β-Glucuronidase activity in 20,000 *g* supernatant is expressed as μg of phenolphthalein liberated after 30 min incubation per 4·0 ml of the exposure system. Scavengers for active oxygen used in the experiments are VE, DABCO, SO-D, BSA and D-mannitol. Irradiation energies were 0, 0·5, 1·0 and 1·5 J/4 ml at 400 nm. In the case of D-mannitol, the reaction mixture was irradiated with 1·0 J/4 ml at 400 nm and the final concentrations of D-mannitol were 0, 12·5 and 125 μM. It was observed that the production of β-glucuronidase activity was inhibited by

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TABLE 2. Lysosomal enzyme activity released after irradiation at 400 nm

| | Acid-phosphatase (nitrophenol μmol/30 min/4ml) | | | β-glucuronidase (phenolphthalein μg/30 min/4ml) | | |
|---|---|---|---|---|---|---|
| HP concentration (M) | $1\cdot9\times10^{-4}$ | $6\cdot2\times10^{-5}$ | 0 | $1\cdot9\times10^{-4}$ | $6\cdot2\times10^{-5}$ | 0 |
| Irradiation (3·0 J/4 ml) (mean ± s.d.)* | 4·36 ± 0·15 | 2·01 ± 0·12 | 1·05 ± 0·14 | 305 ± 23 | 145 ± 17 | 81 ± 12 |
| Non-irradiation (mean ± s.d.)* | 1·03 ± 0·05 | 1·03 ± 0·06 | 0·96 ± 0·07 | 84 ± 11 | 76 ± 9 | 75 ± 8 |
| Student's '*t*'-test | $0{\cdot}001>P$ | $0{\cdot}001>P$ | $0{\cdot}5>P>0{\cdot}4$ | $0{\cdot}001>P$ | $0{\cdot}001>P$ | $0{\cdot}6>P>0{\cdot}5$ |

* n = 5.

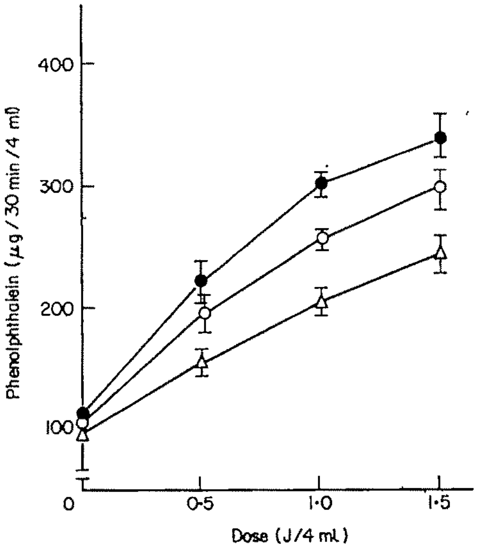


Fig.4.

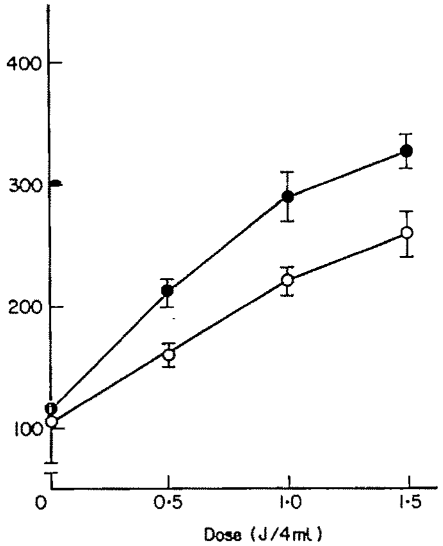


Fig.5.

FIGURE 4. Effect of VE on the release of β-glucuronidase from lysosomal fraction following 400 nm exposure in the presence of HP. A final concentration of VE in irradiation sample of 4·0 ml was 0 (●), 1·25 (○), 12·5 (△) μM, respectively. HP = $1\cdot9\times10^{-4}$ M; n = 3.

FIGURE 5. Effect of DABCO on the release of β-glucuronidase from lysosomal fraction following 400 nm exposure in the presence of HP. The concentration of DABCO was 0 (●) and 125 (○) μM. HP = $1\cdot9\times10^{-4}$ M; n = 3.

39% ($P<0{\cdot}05$) in irradiated sample with 12·5 μM D-mannitol, and by 55% ($P<0{\cdot}01$) in that with 125 μM D-mannitol; expressed as a percentage of the scavenger-free samples. Dose-responses of various scavengers on the β-glucuronidase release from the lysosomal fraction following 400 nm irradiation in the presence of $1\cdot9\times10^{-4}$ M HP are listed in Table 3. All the scavengers, except for 2·5 μg of BSA, prevented significantly the release of β-glucuronidase into supernatant, and the degree of prevention was in proportion to the concentration of the scavengers added.

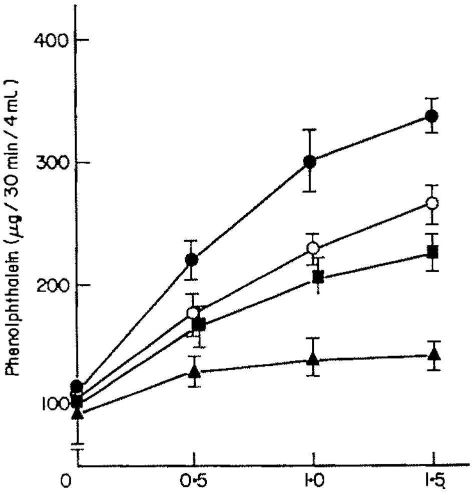


Fig. 6.

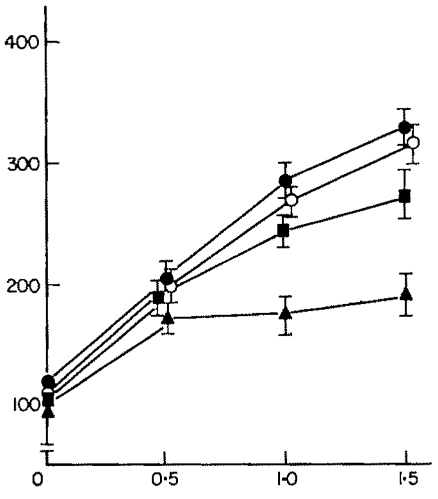


Fig. 7.

FIGURE 6. Effect of SO-D on the release of β-glucuronidase from lysosomal fraction following 400 nm exposure in the presence of HP. Using 2700 units SO-D/mg protein/mg solid, a final value of SO-D in irradiation sample of 4·0 ml was 0 (●), 6·75 (○), 67·5 (■), 675 (▲) units, respectively. HP = $1{\cdot}9\times10^{-4}$ M; n = 3.

FIGURE 7. Effect of BSA on the release of β-glucuronidase from lysosomal fraction following 400 nm exposure in the presence of HP. Using BSA dissolved in a small quantity of physiological saline, finally exposure sample of 4·0 ml contained 0 (●), 2·5 (○), 25 (■), 250 (▲) μg of BSA, respectively. HP = $1{\cdot}9\times10^{-4}$ M; n = 3.

TABLE 3. β-glucuronidase activity released in the supernatant with or without scavengers. The lysosomal fraction was mixed with $1{\cdot}9\times10^{-4}$ M HP and irradiated with 1·5 J/4 ml at 400 nm

|  | Scavenger | Activity (phenolphthalein μg/30 min/4 ml) | Student's '*t*'-test (compared with no scavenger system, respectively) |
|---|---|---|---|
| VE (μM) | 0 | 341 ± 19 | — |
|  | 1·25 | 297 ± 18 | $0{\cdot}05 > P > 0{\cdot}01$ |
|  | 12·5 | 245 ± 16 | $0{\cdot}01 > P > 0{\cdot}001$ |
| DABCO (μM) | 0 | 326 ± 12 | — |
|  | 125 | 259 ± 22 | $0{\cdot}01 > P > 0{\cdot}001$ |
| SO-D (units) | 0 | 337 ± 13 | — |
|  | 6·75 | 264 ± 16 | $0{\cdot}01 > P > 0{\cdot}001$ |
|  | 67·5 | 225 ± 14 | $P < 0{\cdot}001$ |
|  | 675 | 140 ± 10 | $P < 0{\cdot}001$ |
| BSA (μg) | 0 | 330 ± 15 | — |
|  | 2·5 | 314 ± 16 | $0{\cdot}3 > P > 0{\cdot}02$ |
|  | 25 | 273 ± 21 | $0{\cdot}05 > P > 0{\cdot}01$ |
|  | 250 | 192 ± 18 | $P < 0{\cdot}001$ |

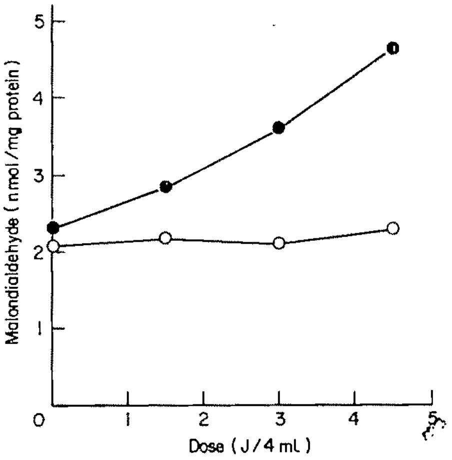


FIGURE 8. Malondialdehyde value in the rat liver homogenate for 400 nm exposure with or without HP. A final concentration of HP was $1\cdot9\times10^{-4}$ M (●) and 0 M (○). The protein concentration in the homogenate was 776 $\mu$g/ml. The MDA value was the average of triplicate determination.

*Lipoperoxide formation in rat liver homogenate following 400 nm exposure in the presence of HP*

The results are shown in Fig. 8. Lipoperoxide formed by 400 nm exposure is expressed as nmol of MDA per mg protein. In preliminary experiments, the MDA value by irradiation was maximum when the homogenate was diluted twenty-four times, the protein concentration of the diluted homogenate being 776 $\mu$g/ml. Dose-response curves of 400 nm irradiation on the formation of MDA in the liver homogenate with and without HP are shown in Fig. 8. The MDA value increased with the increase of the irradiation energy. However, such increase of the MDA was not observed in the absence of HP. The difference between MDA value with HP and that without HP was at a maximum after 4·5 J/4 ml irradiation ($P<0\cdot001$).

## DISCUSSION

In general, molecular oxygen is in triplet state ($^3O_2$), but most chemical compounds are in singlet state, and the reaction between the triplet state oxygen and singlet state chemicals seldom happens. Therefore, non-specific oxygen peroxidation of subcellular organelles is due mostly to: (1) superoxide anion radical ($O_2^-$); (2) hydrogen peroxide ($H_2O_2$); (3) singlet oxygen ($^1O_2$); and (4) hydroxyl radical (OH·) generated by $H_2O_2$ or $O_2^-$. When unsaturated fatty acids are peroxidized by $^1O_2$ or OH·, hydroperoxide is generated (Fig. 9). The hydroperoxide is also regarded as active oxygen because this induces further lipid peroxidation by radical chain reaction (Asada, 1978).

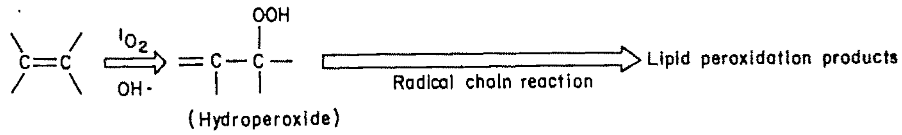
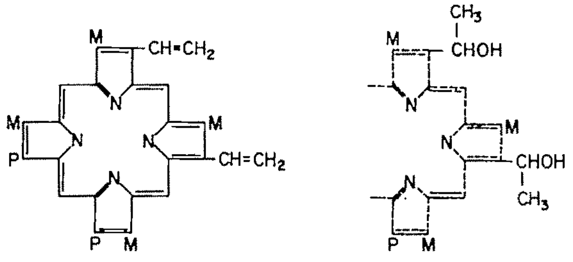


FIGURE 9. Lipid peroxidation by active oxygen (Asada, 1976).

HP, a water-soluble tetrapyrrole, is a synthetic porphyrin which is not known to be produced in living organisms. It differs from protoporphyrin only in the hydration of two vinyl groups to form hydroxyethyl groups (Fig. 10). It is clear that HP causes clinical erythema, oedema and erosion when administered to the skin (Hausmann, 1911; Lipson & Baldes, 1961; Zalar *et al.*, 1977), in addition to degeneration of epidermis, dilatation of vessels and hyperaemia on histopathological examination.



Protoporphyrin IX Haematoporphyrin IX

FIGURE 10. Structural formulas of protoporphyrin and haematoporphyrin (Zalar *et al.*, 1977). P = propionic acid; M = methyl.

Magnus, Porter & Rimington (1959) reported that the action spectrum of the skin disorders in porphyria was at 400 nm and oedema, bleeding and vesicles were produced by 400 nm irradiation in the skin of porphyria cutanea tarda. In summarizing the results of the present experiments, levels of lysosomal enzymes released into the supernatant were significantly high when exposed to 380–420 nm in the presence of HP, but there were no differences in the enzyme levels between 380, 390, 400 and 410 nm irradiation (Table 1).

A photosensitizer such as HP is excited by light, which is absorbed and the excitation energy is then transferred from the HP into molecular oxygen to form the excited singlet state of oxygen (Fig. 11). Whether or not $O_2^-$ is produced has not been investigated yet. Whether or not NBT is reduced when HP is irradiated with 400 nm was investigated. NBT was not reduced in this reaction mixture so it can be assumed that $O_2^-$ is not generated.

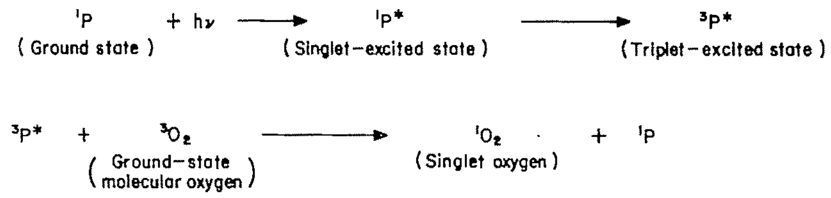

¹P + hν
( Ground state )
¹P*
( Singlet-excited state )
³P*
( Triplet-excited state )
³P* + ³O₂
( Ground-state molecular oxygen )
¹O₂ + ¹P
( Singlet oxygen )

FIGURE 11. Processes of singlet oxygen production due to photosensitizer ($^1P$) and photoenergy (hν) (Asada, 1976).

On the other hand, it has been suggested that when HP is excited with 400 nm, $^1O_2$ may be generated at the last stage as shown in Fig. 11. If $^1O_2$ is generated, it may possibly peroxidize further lipids as indicated in Fig. 9. When the lipids are peroxidized, membranes which consist of lipids and proteins undergo a physical change and are finally destroyed. The phenomenon of photohaemolysis which is applied to the diagnosis of erythropoietic protoporphyria is suggested to be caused by production of small pores on red blood cell membranes resulting from the action of $^1O_2$ generated in the presence of protoporphyrin and light (Harber, Fleischer & Baer, 1964; Goldstein & Harber, 1972).

The effects of various scavengers for active oxygen on the lysosomal membrane are shown in Figs 4–7. The release of lysosomal enzyme was prevented by addition of VE (Fig. 4). It is said that an antioxidant such as VE not only stops the radical chain reaction, but scavenges active oxygen (Asada, 1976). Watabiki & Ogawa (1975) observed an anti-inflammatory effect of VE ointment in mouse skin which was irradiated with 254 nm. Prevention of enzyme release with DABCO, the scavenger for $^1O_2$, was observed although we have not in fact determined the formation of $^1O_2$, so that $^1O_2$ is assumed to have been generated. The effect of DABCO was similar to that of VE.

Since lysosomal fractions used in this series of experiments may contain other organelles such as mitochondria besides lysosomes, $O_2^-$ may also be produced secondarily from other sources (Asada, 1976), though $^1O_2$ was seemingly only produced by HP and 400 nm. OH· may also be generated by Harber-Weiss reaction ($H_2O_2+O_2^-+H^+ \rightarrow H_2O+{}^1O_2+OH\cdot$). Therefore, it is assumed that SO-D and scavenger for OH· might be effective in our experimental system. Release of lysosomal enzymes was prevented with D-mannitol which is a scavenger for OH·. SO-D catalyses the dismutation of $O_2^-$, and therefore, will inhibit the production of $^1O_2$ and OH·. This mechanism is very important for the protection of living organisms. In our experiment, SO-D showed the preventive effect on the enzyme release. BSA, which probably does not possess a scavenger function, also prevented the release of lysosomal enzyme when 25 $\mu$g or more of BSA was added (Fig. 7 and Table 3). Therefore it is assumed that SO-D not only catalyses $O_2^-$ produced secondarily but also reacts as protein, since protein such as BSA eliminates lipoperoxide formation (Asada, 1976).

The mechanism of skin photosensitivity in porphyria is considered from these *in vitro* experimental results as follows. Cell membranes including lysosomal membranes will be destroyed by porphyrin and 400 nm irradiation, many lysosomal enzymes are released into inter- and/or intra-cellular space, then chemical mediators by which various skin changes develop will be produced. Rimington *et al.* (1967) reported that histamine was probably one of the mediators of urticaria formation produced with 400 nm irradiation to a patient with porphyria.

Cell degeneration or necrosis produced by HP and 400 nm irradiation is probably due not only to the digestion of membrane by released enzymes from lysosomes but also to hydroperoxide and lipid peroxidation products thus formed. Lipoperoxide finally turns into MDA that crosslinks both ribonuclease and other enzymes, and diminishes their activities (Chio & Tappel, 1969). The MDA value was in proportion to the irradiation energy of 400 nm with HP in our experiment (Fig. 8). Meffert, Diezel & Sõnnicksen (1976) reported that products of lipid peroxidation (MDA) were increased after UV exposure on chronically sun-exposed areas as well as with advancing age. It is assumed that measurement of MDA in skin of porphyria might offer a clue for the mechanism of photosensitivity in porphyria.

## ACKNOWLEDGMENTS

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# Psychiatric aspects of dermatitis artefacta

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## SUMMARY

Fifty patients with dermatitis artefacta were investigated psychiatrically over several years and twenty-six of these were seen independently by a clinical psychologist.

The psychological investigations suggest a personality structure characterized by inward-looking self-centred attitudes leading to increasing isolation.

The psychiatric investigations suggest a background of emotional disturbances during the formative years and in later life often resulting in feelings of isolation and insecurity. The onset of dermatitis artefacta is very frequently related to definable precipitating events which vary according to age and life situations. The visible skin lesions can be understood as an attempt at non-verbal communication subserving an appeal function.

Therapeutic methods and prognosis are discussed in some detail.

The monographs by Wittkower & Russell (1953) and by Whitlock (1976) on disorders of the skin and their relation to psychological problems have dealt with dermatitis artefacta (DA) in the wider setting of dermatoses in general. Michelson (1945), Obermeyer (1955), Waisman (1965) and Musaf (1969) considered questions of motivation and purpose and the place of DA in the group of self-inflicted disease in general. Kidd & Watt (1967) commented on differences in the psychological background of patients who place their lesions in visible and concealed areas. A number of investigations have dealt with the environmental conditions of patients with DA (Preston, 1969; Tobias, 1969; Shanon, 1970). Therapeutic problems were discussed by Aleck (1968) and Baer & Kuypers (1973). The papers by Lyell (1972, 1976) and Sneddon & Sneddon (1975) surveyed background, presentation, the course of the condition and the prognosis in considerable detail.

## MATERIALS AND METHODS

This paper is based on fifty patients seen between 1961 and 1978 (Table 1). The first twenty-six patients were investigated by the author together with Dr J. Kamieniecki, former Clinical Psychologist at Mapperley Hospital, Nottingham, the remainder by the author alone. All patients were referred by dermatologists of Nottingham General Hospital with the diagnosis of DA. Circumstances made it

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impossible to compare this group with other patients suffering from skin diseases affecting parts of the body commonly covered and which were not self-induced.

TABLE 1. Number and age of patients

| | No. | Mean age (years) | Age range (years) |
|---|---|---|---|
| Male | 6 | 33·4 | 11–62 |
| Female | 44 | 27·1 | 9–70 |

For the psychological assessment of adult patients the following tests were used: Mill Hill Vocabulary, Raven's Advanced Progressive Matrices (shortened version), Maudsley Personality Inventory (Eysenck) and Rorschach Inkblot Personality Projection. For some of the school children the Revised Stanford-Binet, Koh's Block Design and Wechsler Intelligence Scale for Children were used.

## RESULTS

The clinical evaluation of the patients was based on non-structured interviews supplemented by information from medical records, social workers' reports and information obtained from relatives and occasionally from friends. The results of the psychological investigations usually became available after the second or third interview. All patients were seen at a Dermatological out-patient Department with the exception of three school children who attended a school clinic; psychological assessments of these were supplied by educational psychologists.

### *Occupation*

All patients under 16 attended school. Four of the five males over 16 were gainfully employed. The thirty-seven females over 16 were either employed, looked after households or did both.

### *General state of health*

There was no incapacitating disease at the time of investigation but the somatic complaints shown in Table 2 were noted.

TABLE 2. General state of health of patients

| Sex | Age | Diagnosis |
|---|---|---|
| M | 11 | Familial cataract* |
| M | 43 | Diabetes mellitus |
| M | 57 | Shortening of leg after osteomyelitis |
| M | 62 | Severe deafness of unknown origin, lumbar spondylosis* |
| F | 11 | Congenital hypospadias, corrected by surgery |
| F | 15 | Post-traumatic brain damage |
| F | 18 | Diabetes mellitus with necrobiosis lipoidica |
| F | 39 | Psychomotor epilepsy |
| F | 49 | Rachitic dwarfism |
| F | 50 | Otosclerotic deafness* |

* These patients suffered from sensory defects which may have affected communication thus causing a moderate degree of isolation.

*Psychological tests*

Intelligence tests on the whole showed a fairly typical Gaussian distribution to which three school children added a slight shift to the left (Table 3). The results of the Maudsley Personality Inventory are shown in Table 4.

*Rorschach inkblot personality projection*

The general picture was one of inward looking persons, in many respects self-centred, who find it difficult to make easy and satisfactory contact with others. Their interests are limited and restricted in a rigid and formalized manner. Some showed emotional immaturity, others were decscribed as emotionally flat. Few of the patients showed a warm-hearted out-going personality capable of enjoying a well established relationship with others.

NB. Two of the twenty-six patients were unable to complete all the items of the MPI and Rorschach tests. They are not included in Table 4.

TABLE 3. Results of intelligence tests

| Intelligence | IQ range | No. |
|---|---|---|
| Average | 90–109 | 10 |
| Dull normal | 80–89 | 8 |
| High average | 110–119 | 8 |
| ESN (M) | 50–75 | 3 |

TABLE 4. Maudsley Personality Inventory

| | No. |
|---|---|
| Extraversion | 13 |
| Introversion | 10 |
| Equal score | 1 |
| Neuroticism normal range | 14 |
| Hysterical features | 4 |
| Dysthymic features | 5 |
| 'Non-descript pattern' | 1 |

*Psychiatric diagnoses*

For the diagnoses the system described in the Glossary of Mental Disorders (HM Stationary Office 1968) was adopted as far as possible (Table 5).

*Comments.* Half the patients presented with neurotic disorders and uni-polar depression. Only two were true hysterics: an adolescent girl who had inflicted a burn on her left forearm and showed post-traumatic anaesthesia and amnesia; a menopausal woman with a life-long history of neurotic problems including an episode of pseudocyesis. The other main group, personality disorders, included three obsessional, two paranoid, and one each immature, cyclothymic, inadequate persons as well as one adolescent delinquent. Conduct disorders were diagnosed in four school children, three of whom were

TABLE 5. Psychiatric diagnoses of patients

| Diagnosis | No. | Diagnosis | No. |
|---|---|---|---|
| Neurosis, chronic | 10 | Manic-depressive psychosis | 1 |
| Neurotic reaction | 7 | Brain damage, post-traumatic | 1 |
| Personality disorder | 9 | Temporal-lobe epilepsy | 1 |
| Depression, uni-polar | 8 | Deterioration, involutional | 1 |
| Conduct disorder | 5 | Deterioration, senile | 1 |
| Stress reactions | 4 | No diagnosis | 2 |

of borderline intelligence; the stress reactions were found in adolescents and young adults due to difficult home and working situations.

### *Background to personality development*

A systematic survey of circumstances which in part at least could be considered responsible for shaping the personality was possible in thirty-two patients (Table 6). As this was essentially a matter of history taking the usual difficulties, particularly in the higher age groups, were encountered.

TABLE 6. Background abnormalities (thirty-two patients)

| | No. |
|---|---|
| Parental home | 21 |
| Chronic ill-health | 4 |
| Matrimonial and/or work problems | 5 |
| Sibling rivalry | 1 |
| Precocious somatic development | 1 |

In the largest group of twenty-one patients the parental home appeared of decisive importance: parental dysharmony leading to separation and divorce, faults in the parent/patient relationship, and bereavement all affecting mainly the younger age groups, while matrimonial and work problems were of importance in older age groups. This is obviously an over-simplified picture. In three patients somatic disease played an important role (congenital hypospadias, post-traumatic brain damage, temporal lobe epilepsy).

### *Precipitating events*

Such events being of comparatively recent date were described more often and usually in great detail (Table 7).

Age plays an important role: out of ten children of school age experiences at school were operative in six; developmental, sexual, and work problems acted as precipitants from puberty to the mid-twenties in ten out of nineteen cases, while later on work, family life, illness and bereavement were responsible with increasing frequency. Again very often more than one factor was present and the choice of the most important is, to some extent arbitrary.

TABLE 7. Recent precipitating events in forty-three patients

| | No. |
|---|---|
| Developmental, sexual, matrimonial | 11 |
| Illness, accident, bereavement | 8 |
| Family problems | 8 |
| Work problems | 8 |
| School problems | 6 |
| Social isolation in deaf person | 1 |
| First contact with the Law | 1 |

## DISCUSSION

The method outlined above for the investigation of patients with DA has been found useful though rather time-consuming, but it helped to understand how DA came to be adopted as a method of appealing for attention and help. It was also useful to suggest therapeutic strategies as well as to formulate a prognosis.

Drugs, mainly minor tranquillisers, anti-depressants and night sedatives, can be useful in the initial stages when anxiety, depression and insomnia may be troublesome. As one is dealing with a potentially chronic disease in which relapses are common, drugs should be used sparingly and only for short periods.

'Manipulation of the environment' in a wide sense can be tried. This applies mainly to young school children especially to those with learning problems. Older children and adolescents may need advice and help with training and employment. Involvement of parents and guardians in these younger age groups is essential. However, family therapy in the true sense of the term was not attempted. In the older age groups Welfare and Social Services Departments may have to be asked for advice and help.

An important function of DA seems to be an appeal for help. Therefore, the main precondition for the psychiatric approach in the narrower sense was the establishment of understanding and trust between patient and therapist. On a superficial level this aim was achieved in most cases. To reach deeper levels was more difficult. As treatment progressed many psychological mechanisms became obvious: the desire for self-punishment to achieve relief from tensions caused by fear and guilt; exhibitionistic-hysterical symptoms; strategems for testing out the tolerance of persons including the psychiatrist. Transference situations did develop which required therapeutic sessions for long periods. These are some of the aspects which have been described in the literature with varying emphasis as *the* responsible psychological mechanism. Seen as different forms of appeal they make sense; they help doctor and patient to understand each other, the first step towards an attitude which will permit treatment.

The prognosis varied between the extremes of recovery almost before the first attendance of the patient and persistence of DA for decades. In the first group there was a patient of 50 who had been suffering from increasing deafness for years and who in the past had refused to use a hearing aid. DA developed when she moved to Nottingham on the transfer of her husband to a new job. The move inevitably caused an increase of social isolation and after a short while she began to use her hearing aid. At her first clinic attendance the DA showed signs of improvement leading to full recovery in a few months.

An example of the second group is another female patient now aged 35 whose DA began at 7 years of age, after several years of hospitalization and other types of absence from a very disturbed parental home. She attended with interruptions for about 12 years from 1964 to 1976, presenting a depressive-paranoid-aggressive symptomatology. There were only very short periods of improvement. Most cases lie between these two extremes.

Young children on the whole have a better prognosis because growing up will help them to deal with their own problems, and the passage of time helps the parents to overcome their own anxieties. The prognosis has to be guarded in patients in their involutional years and in old age, but the two examples described above show how difficult it is to generalize. The quality of the personality and the stability of the life situation play an important and at times decisive role. This has been emphasized in particular by Pao (1969), Lyell (1972, 1976) and Sneddon & Sneddon (1975).

Finally DA has to be seen as only a small segment of the spectrum which starts with patients who rub and scratch themselves habitually and apparently without deeper meaning, and which ends with self-destruction by suicide.

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# Effects of corticosteroids on the proliferation of normal and abnormal human connective tissue cells

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## SUMMARY

Four corticosteroids were tested *in vitro* for effect on the proliferation of four strains of fibroblasts from scleroderma skin, four strains from normal adult skin and four strains of rheumatoid synovial cells.

Significant effects on fibroblasts occurred only at the highest steroid concentration tested (10 $\mu$g/ml) where the inhibitory ranking of the steroids was clobetasol propionate > clobetasone butyrate > betamethasone valerate > hydrocortisone. Hydrocortisone and betamethasone valerate stimulated proliferation of two normal strains, had no certain effect on the scleroderma group, and inhibited growth of synovial cells. Clobetasone butyrate and clobetasol propionate inhibited growth of all cells. All four steroids substantially reduced acid mucopolysaccharide secretion by scleroderma fibroblasts.

These results suggest that fibroblasts from normal and abnormal skin show only small differences in their responses to corticosteroids *in vitro*, but contrast sharply with the mouse L-929 fibroblasts previously used in some assays of topical corticosteroid potency.

Berliner & Ruhmann (1967) suggested that the inhibitory effects of corticosteroids on the proliferation of mouse L-929 fibroblasts *in vitro* were related to the anti-inflammatory capacity of the same steroids applied to human skin *in vivo*. The relationship, though useful, appears to be fortuitous. It is now clear that L-929 fibroblasts are far more sensitive to inhibition by corticosteroids than fibroblasts from normal human skin, which are not necessarily inhibited at all (Castor, 1962; Kirk & Mittwoch, 1977; Runikis, McLean & Stewart, 1978; Priestley, 1978), but it is still possible that L cells model the response of some abnormal human fibroblasts (Kirk & Mittwoch, 1977). In this paper we examine the behaviour of cells recently isolated from abnormal human skin and connective tissue. We report the effect of four corticosteroids on fibroblasts cultured from scleroderma skin, on fibroblasts from normal skin of persons of comparable age, and on rheumatoid synovial cells.

## MATERIALS AND METHODS

### Cell strains

Skin fibroblast strains (designated HSF8, 10, 11, 12) from normal adults (average age 45) were obtained from the Royal Hospital for Sick Children, Edinburgh. Strains of rheumatoid synovial cells (designated RA1, 2, 5 and 6) were grown from synovia removed at elbow or knee synovectomy from

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rheumatoid arthritis patients, average age 60, as previously described (Priestley & Brown, 1979). In the scleroderma group, strains SD3 and SD6 were derived from patients with systemic sclerosis attending this Department (Priestley & Brown, 1979) and two further strains (SD9: generalized morphoea, age 50; SD10: systemic sclerosis, age 56) were kindly provided by Dr F.M.Pope. Tests were performed in passages 5–17.

### *Culture*

Cells were grown in Nunc plastic flasks in Dulbecco-Eagle medium with 10% fetal calf serum (Gibco-Biocult Ltd, Paisley) and antibiotics at 37°C as previously described (Priestley & Brown, 1978, 1979).

### *Proliferation tests*

The method was slightly different from that used in our earlier study of corticosteroids (Priestley, 1978). On day 0, twenty four flasks (growth area 25 $cm^2$) were seeded with aliquots of about $1 \times 10^5$ cells. The medium was replaced on day 1. On day 3 four flasks were withdrawn for cell counts; the cells were washed in phosphate buffered saline, released from the flask with trypsin-versene and counted in a Coulter Counter. Disagreement in duplicate counts on each sample was less than 1%. The remaining flasks were divided into five groups of four, allowing treatment with each of the four corticosteroids at 10 μg/ml, and a second group of four control cultures. The steroids were dissolved in 1:1 dimethyl sulphoxide-propylene glycol for addition to culture medium; control cultures received 0·1% of this vehicle only. On day 4 all media were renewed. On day 6 the cells in the cultures were counted. The increase in cell number in steroid media was expressed as a percentage of the mean increase in controls between days 3 and 6. Final cell numbers were about $1 \times 10^6$ fibroblasts and $0{\cdot}7 \times 10^6$ synovial cells.

In other experiments the flasks were treated with different concentrations (0·0001–10 μg/ml) of one corticosteroid to obtain dose-response curves. These experiments used a total of thirty-two flasks, four for each concentration of steroid.

### *Acid mucopolysaccharide assay*

Medium collected on day 6 of a proliferation test on SD6 cells was prepared for acid mucopolysaccharide assay by dialysis, concentration and protein precipitation, as previously described (Priestley & Brown, 1978), and uronic acid in protein-free supernatants was measured using *m*-hydroxydiphenyl reagent (Blumenkrantz & Asboe-Hansen, 1973) with a glucuronolactone standard. Material in unused medium was also assayed for calculation of output per culture, and output per $10^7$ cells was calculated using day 6 cell counts.

## RESULTS

The effect of the corticosteroids at 10 μg/ml on cell proliferation is summarized in Table 1. As in earlier tests on foreskin fibroblasts from normal juvenile donors (Priestley, 1978) there was a contrasting effect on the normal cells: proliferation of HSF8 and HSF10 strains was stimulated by hydrocortisone and betamethasone valerate, while clobetasone butyrate had no significant effect or inhibited growth and clobetasol propionate inhibited proliferation in all four strains. Differences in the response of the individual strains within the group were common and often statistically significant: for instance all four pairs of figures for HSF10 and HSF12 differ significantly ($P<0{\cdot}01$), as do those for HSF8 and HSF10 ($P<0{\cdot}05$).

The same pattern of differences between strains and between steroids is present in the data for the scleroderma group: hydrocortisone stimulated growth very slightly in SD6 only, betamethasone valerate inhibited growth in SD3 and SD10 and clobetasone butyrate and clobetasol propionate again inhibited the proliferation of all four cell strains. Here the responses of SD3 and SD6 for each steroid contrast significantly ($P < 0·02$). The rheumatoid synovial strains were generally inhibited more than either the normal or scleroderma skin fibroblasts ($P < 0·001$ in pooled HSF versus RA data for each steroid). There was no stimulation with hydrocortisone and in the final analysis of pooled data from the four synovial strains all steroids produced significant inhibition.

The use of pooled data can conceal significant effects, for instance with betamethasone valerate in HSF strains, but allows us to compare the performance of the four steroids in general terms. It is clear that hydrocortisone gave the least inhibition and clobetasol propionate the most, with clobetasone butyrate and betamethasone valerate intermediate. Although results for these last two compounds overlap, clobetasone butyrate was more inhibitory in nine cell strains so that the final ranking is clobetasol propionate > clobetasone butyrate > betamethasone valerate > hydrocortisone.

Dose–response curves for two scleroderma strains, SD3 and SD6 fibroblasts, are combined in Fig. 1a, and Fig. 1b shows the response in RA6 cells. Although there is a general downward trend in the curves in Fig. 1a, only the values at 10 μg/ml reach statistical significance. The effect is much clearer in Fig. 1b where the ranking of the steroids by inhibitory capacity, clobetasol propionate > clobetasone butyrate > betamethasone valerate > hydrocortisone, is the same at all concentrations and regression analysis leaves the significance of the dose–response in no doubt.

At 10 μg/ml all four steroids substantially reduced acid mucopolysaccharide secretion by SD6

TABLE 1. Effect of corticosteroids at 10 μg/ml on proliferation of normal and abnormal connective tissue cells. Results shown for each cell strain are means ±s.e.mean (n = 4)

| Cells | Controls | CB | BMV | HC | CP |
|---|---|---|---|---|---|
| HSF 8 | 100±2 | 95±1 | 120±1‡ | 133±2‡ | 61±4‡ |
| HSF10 | 100±4 | 81±2† | 114±2* | 140±4‡ | 64±2‡ |
| HSF11 | 100±7 | 85±4 | 84±6 | 108±6 | 47±1‡ |
| HSF12 | 100±8 | 64±3† | 91±4 | 94±2 | 50±2‡ |
| Pooled HSF mean (n = 16) | 100±3 | 81±3‡ | 102±4 | 118±5† | 55±2‡ |
| SD 3 | 100±3 | 57±3‡ | 89±3* | 95±5 | 40±4‡ |
| SD 6 | 100±4 | 77±5† | 107±5 | 111±1* | 69±5‡ |
| SD 9 | 100±2 | 76±3‡ | 105±4 | 103±3 | 65±4‡ |
| SD10 | 100±5 | 81±7* | 75±6* | 91±9 | 43±2‡ |
| Pooled SD mean (n = 16) | 100±2 | 73±3‡ | 94±4 | 100±3 | 54±4‡ |
| RA1 | 100±2 | 61±2‡ | 68±6† | 92±1* | 50±2‡ |
| RA2 | 100±5 | 78±3* | 68±6† | 92±5 | 39±3‡ |
| RA5 | 100±3 | 51±2‡ | 66±3‡ | 79±2† | 35±2‡ |
| RA6 | 100±2 | 72±4‡ | 73±3‡ | 81±4† | 44±1‡ |
| Pooled RA mean (n = 16) | 100±2 | 65±3‡ | 69±2‡ | 86±2‡ | 42±2‡ |

'*t*'-test comparison with controls: *$P < 0·05$ †$P < 0·01$ ‡$P < 0·001$. Cell strains: HSF = normal skin fibroblasts; SD = scleroderma skin fibroblasts; RA = rheumatoid synovial cells. CB = clobetasol butyrate; BMV = betamethasone valerate; HC = hydrocortisone; CP = clobetasol propionate.

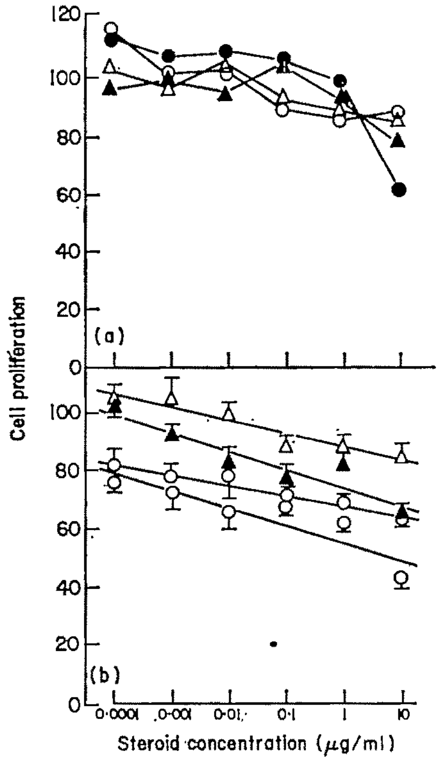

FIGURE 1. Effect of corticosteroids on the proliferation of SD3 and SD6 fibroblasts ((a) n = 8) and RA6 rheumatoid synovial cells ((b) n = 4, mean ± s.e.mean) (controls = 100). The steroids were clobetasone butyrate (○—○); hydrocortisone (△—△); betamethasone valerate (▲—▲); clobetasol propionate (●—●). Regression coefficients in (b): hydrocortisone $r = -0\cdot66$, $P < 0\cdot001$; betamethasone valerate $r = -0\cdot78$, $P < 0\cdot001$; clobetasol butyrate $r = -0\cdot69$, $P < 0\cdot001$; clobetasol propionate $r = -0\cdot71$, $P < 0\cdot001$.

fibroblasts (Fig. 2) both per culture and as calculated output per $10^7$ cells. In a single dose-response experiment with RA6 cells, clobetasol propionate gave a clearly dose-related inhibition of acid mucopolysaccharide secretion ($r = -0\cdot84$, $P < 0\cdot001$ at 26 d.f.) with 80% inhibition of secretion per $10^7$ cells at 10 μg/ml.

## DISCUSSION

The responses of the normal fibroblasts in Table 1, cells grown from the forearms of adult donors, resemble those of juvenile foreskin fibroblasts exposed to the same four steroids at the same concentration (Priestley, 1978). Although clobetasol butyrate produced less inhibition than previously, the ranking of the steroids is identical. This shows that age of donor is not critical in this assay, and also removes the possibility that because genital skin fibroblasts are hypersensitive to testosterone (Pinsky *et al.*, 1972) they are therefore an invalid model for testing corticosteroids. Both these findings support the recent report by Ponec *et al.* (1977a).

Electronic cell counting is extremely accurate, but some part of the considerable differences between cell strains within the groups in Table 1 may arise from undue variation in controls (which

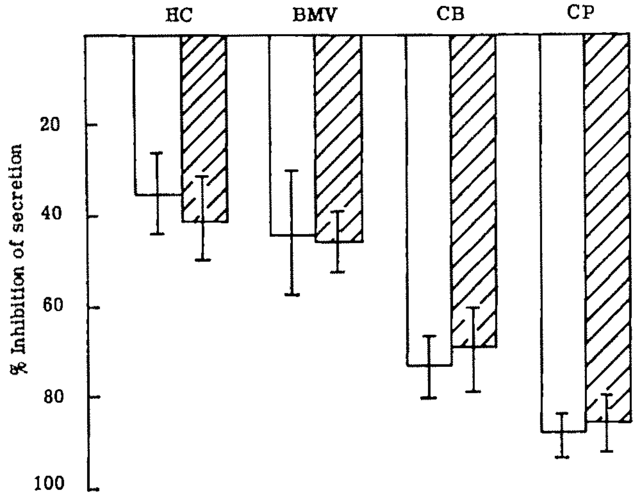

FIGURE 2. Inhibition of acid mucopolysaccharide secretion by SD6 scleroderma fibroblasts with 10 μg/ml corticosteroids. Values are means ± s.e.mean (n = 4); those for secretion per $10^7$ cells are all significantly different from controls ($P < 0{\cdot}01$; '*t*'-test). HC = hydrocortisone; BMV = betamethasone valerate; CB = clobetasol butyrate; CP = clobetasol propionate. Open columns: uronic acid secretion per culture; hatched columns: uronic acid secretion per $10^7$ cells.

would affect all steroid results in the experiment) or from the use of only four cultures per steroid. Where we have repeated experiments, however, or compared results for the same cell strain in dose–response experiments at the same concentration of steroid (10 μg/ml), there was good agreement (Table 1, Fig. 1). Some cell strains have distinctive characteristics: over many experiments SD3 fibroblasts consistently grow more slowly than SD6, but secrete almost twice as much mucopolysaccharide (SD3 109±5 (s.e.mean) μg uronic acid/48 h/$10^7$ cells; SD6 60±4 μg, $P < 0{\cdot}001$ at 22 d.f.), and devote more of their total protein synthesis to collagen. Similar consistent, and possibly genetic, differences in growth rate and uronic acid output distinguish some strains in the rheumatoid group. The normal strains HSF8–12 have not been examined in this detail but foreskin fibroblasts (Priestley, 1978; Priestley & Brown, 1978, 1979) also show marked differences in uronic acid secretion. It would not then be surprising if fibroblast strains varied slightly in their response to a particular drug, and this may be a component of the variation in clinical response shown by individual patients. For testing drug responses it is clearly preferable to use several strains.

The differences between normal and abnormal groups of cells in Table 1 must be judged in the context of this variation between normal strains. The scleroderma skin fibroblasts responded differently to two out of four corticosteroids, most notably with an 18% overall difference between hydrocortisone's stimulation of normal cells and its lack of effect in scleroderma cells ($P < 0{\cdot}01$). Effects on rheumatoid synovial cells repeatedly exceeded those in the fibroblasts; probably they resemble normal synovial cells (Fell, 1978) rather than normal skin fibroblasts. The biggest discrepancies were between hydrocortisone's overall 18% stimulation of normal cells and 14% overall inhibition of the synovial group, both small but significant effects, and between betamethasone valerate's lack of effect on normal cells and 31% inhibition of synovial cells. The small scale of these differences clearly shows that these groups of human cell strains resemble one another in their response to corticosteroids. Mouse L-929 fibroblasts are no longer acceptable as a model for normal human cells (Ponec *et al.*, 1977a; Runikis *et al.*, 1978; Priestley, 1978; Pratt, 1978) because of the immense contrast in their steroid susceptibility (put at $10^3$ or $10^4$ by Ponec *et al.*) but L-929 fibroblasts have been suggested as a model for certain abnormal human mesenchymal cells (Kirk & Mittwoch, 1977). Our data give this suggestion no support, and the responses of the same cells to griseofulvin, para-aminobenzoate, D-penicillamine and salicylate (Priestley & Brown, 1979, and unpublished data) have also failed to show a consistent pattern of differences between the cells from normal and abnormal connective tissue.

The stimulation of proliferation with 10 μg/ml hydrocortisone and betamethasone valerate in two normal strains confirms our earlier findings with foreskin fibroblasts (Priestley, 1978). We have never seen stimulation with clobetasone butyrate or clobetasol propionate (although 0·0001 μg/ml concentrations in dose–response experiments (Fig. 1) sometimes give suggestive results), nor with non-steroid drugs tested on the same cells (Priestley & Brown, 1978, 1979, and unpublished data). Several laboratories have now reported glucocorticoid-stimulation of human skin fibroblast growth in varying circumstances, and the list of compounds with this capacity now includes hydrocortisone, hydrocortisone butyrate, betamethasone valerate, triamcinolone acetonide, fluocinolone acetate, desonide, and beclomethasone dipropionate (Castor, 1962; Thrash & Cunningham, 1973; Runikis *et al.*, 1978; Ponec *et al.*, 1977b; Priestley, 1978). The degree of stimulation has seldom exceeded 50%, and the mechanism remains uncertain, though Thrash & Cunningham (1973) suggested that altered acid mucopolysaccharide metabolism may be involved, and Kirk & Mittwoch (1977) showed that treatment provoked earlier entry into the S phase of the cell cycle. Stimulation of confluent cultures (Thrash & Cunningham, 1973) suggests that an extension of the logarithmic growth phase may occur, and stimulation in confluent but not in proliferating cultures (Ponec *et al.*, 1977a, b) would agree with this. However, some of our data are less easily interpreted in this way, for instance, the degree of stimulation of 3–5 day growth (undoubtedly logarithmic) of foreskin fibroblasts matched that seen over 3–7 days (Priestley, 1978). Some studies (Harvey, Grahame & Panayi, 1974) have not recorded any stimulation of growth.

Inhibition of proliferation is a useful indication of the fibroblast's overall response to drugs, implying that total metabolism is affected. However, some functions may be more sensitive to drugs than proliferation. Acid mucopolysaccharide secretion is inhibited when proliferation is stimulated by hydrocortisone (Castor, 1962; Priestley, 1978) and collagen synthesis may be suppressed, e.g. by betamethasone valerate, without inhibiting cell proliferation (Ponec *et al.*, 1977b).

The effects on acid mucopolysaccharide secretion in scleroderma cells (Fig. 2) and in RA6 cells treated with clobetasol propionate, closely matched the inhibition recorded previously in normal foreskin fibroblasts (Priestley, 1978), and the ranking of the steroids was the same. Although clobetasol propionate suppressed collagen synthesis in both normal and scleroderma fibroblasts (Priestley, 1978; Priestley & Brown, 1979) other drugs are not necessarily equally effective in the two types of cells. D-Penicillamine, for instance, reportedly prevents crosslinking of immature collagen in proliferating scleroderma skin, but not in quiescent scleroderma or normal skin fibroblasts (Herbert *et al.*, 1974).

There was good agreement in our experiments on the inhibitory ranking of the four steroids, i.e. clobetasol propionate > clobetasone butyrate > betamethasone valerate > hydrocortisone. The total range of the effects was small, however, compared to the wide differences (almost 2000 fold) seen between hydrocortisone and clobetasol propionate in vasoconstriction tests of corticosteroid potency (Phillips, 1976) or fibroblast inhibition assays on L-929 cells (Berliner & Ruhmann, 1967; Brotherton, 1971). The smaller differences shown here seem to us more compatible with the relatively small differences in clinical performance of different corticosteroids measured, for instance, as minimum treatment times (see, for example, Munro, 1976).

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# Eosinophil chemotactic factor (ECF) in blister fluid of dermatological diseases

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## SUMMARY

The occurrence of low molecular weight (<500 dalton) eosinophil chemotactic factor (ECF) was studied in the blister fluid of patients with various dermatological diseases by an *in vitro* chemotaxis method. After chromatographic separation of the test substance on a small Sephadex G 25 column, ECF was demonstrated in blisters of five patients with bullous pemphigoid, in three patients with systemic drug reactions and in one patient with blisters after contact with primula. ECF was not found in blisters of patients with epidermolysis bullosa and herpes gestationis or in DNCB-induced blisters. Fluid obtained from suction blisters was negative in normal controls and on unaffected skin of patients with atopic eczema, but contained ECF in eczematous areas. The demonstration of ECF in tissue fluid suggests that this factor plays a role in the local accumulation of eosinophils at sites of certain inflammatory reactions.

Although the role of eosinophils is still unclear, these cells are observed in histological sections of a number of dermatological diseases. Several factors are known to attract eosinophils across membranes *in vitro*, including peptides of the complement cascade (C5a) (Ward, Cochrane & Müller-Eberhard, 1965) and products of lymphocytes (Cohen & Ward, 1971; Colley, 1973), mast cells (Kay, Stechschulte & Austen, 1971), basophils (Parish, 1972) and neutrophils (PMN) (Czarnetzki, König & Lichtenstein, 1976a). The factor released from mast cells is called eosinophil chemotactic factor of anaphylaxis (ECF-A), consists of at least two tetrapeptides (Goetzl & Austen, 1975) and is very similar in its physicochemical properties to the eosinophil chemotactic factor (ECF) secreted during phagocytosis by PMN (Czarnetzki, König & Lichtenstein, 1975; Czarnetzki, 1978). In the present paper, the presence of the latter factor(s) in blister fluid of some dermatological diseases associated with eosinophilia has been investigated, in order to obtain information on the *in vivo* role of the low molecular weight ECF in the local attraction of eosinophils.

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## MATERIALS AND METHODS

Fluid was obtained from patients with selected blistering diseases by aspiration with a sterile needle and syringe after cleansing the skin with alcohol. Cells were separated from the fluid by centrifugation at 400 *g* for 15 min at 4°C, and the supernatant was processed immediately or stored at −60°C until use. The cells were stained (Wright's stain) and a differential count was performed. Serum was obtained after allowing venous blood to clot for 1 h at 21°C followed by centrifugation at 3000 *g* for 30 min. Serum was then handled exactly as the blister fluid.

Suction blisters (8 mm diameter) were induced on selected skin areas by placing plastic cups attached to a vacuum pump, adapted from Hellum & Solberg (1977). The negative pressure (300–400 mmHg) was released immediately after the blister had formed in order to avoid bleeding into the site. For the demonstration of ECF, 200 $\mu$l of the test substance were applied on a $14 \times 1{\cdot}5$ cm Sephadex G 25 column and eluted in Tris buffer (pH 7·35, 0·025 M). Blue dextran 2000 (Pharmacia, Uppsala, Sweden), phenol red and vitamin $B_{12}$ (Sigma Chemical Co., Munich, FRG) were used as molecular markers. ECF obtained after phagocytosis of zymosan (König, Czarnetzki & Lichtenstein, 1976) or ECF-A from sonicated human lung fragments (Czarnetzki, König & Lichtenstein, 1976b) served to identify the elution range for low molecular weight ECF. The pattern did not differ for either ECF or ECF-A.

Alternative column fractions were examined for eosinophil chemotactic activity in modified plexiglass chemotaxis chambers with human or guinea-pig eosinophils as indicator cells. Details of this method have been described (Czarnetzki *et al.*, 1976a,b). Results are expressed as the numbers of eosinophils that have migrated completely through a nitro-cellulose filter (Sartorius Membran Filter, GmbH, Göttingen, FRG) in five random microscopic fields at ×450 magnification (Eos/5 HPF). All columns were tested at least in duplicate on separate occasions.

IgE levels were measured by a radioimmune assay (Phadebas-Test-Kit, Pharmacia, Uppsala, Sweden) and kindly performed in the laboratory of Dr K.J. Kalveram.

## RESULTS

In Fig. 1a, a representative elution pattern for PMN-derived ECF on the G 25 column is demonstrated, with peak activity between vitamin $B_{12}$ and phenol red. In Fig. 1b the eosinophil chemotactic activity in fractionated fluid from a suction blister of eczematous skin (atopic dermatitis) is illustrated, the peak being again between vitamin $B_{12}$ and phenol red. The slightly increased activity later on (elution volume 20·3–23·1 ml) was not reproducible. The peak elution between vitamin $B_{12}$ and phenol red was found with all the positive test substances discussed later. In each case, the quantity of ECF in biological material was very low compared to that generated *in vitro* from PMN. Finally, a negative example is illustrated in Fig. 1c. No ECF could be demonstrated in the suction blisters of control subjects.

The data on five patients with untreated bullous pemphigoid are shown in Table 1. The typical clinical picture had been confirmed by a positive direct and indirect antibasement antibody detected by immunofluorescence. ECF was found in serum and blister fluid in each case. Serum IgE levels exceeded those in blister fluid in four of five cases while the reverse relationship was found for percentages of eosinophils. The latter finding goes well with increased levels of ECF in blister fluid compared to serum. When serum and blister fluid were tested in the same assay, the peak ECF activity in blisters generally exceeded that in serum by more than 50%.

Patients with blisters due to various other dermatological diseases were also examined. The results are shown in Table 2. Five patients had blisters due to systemic drug reactions, the cause being

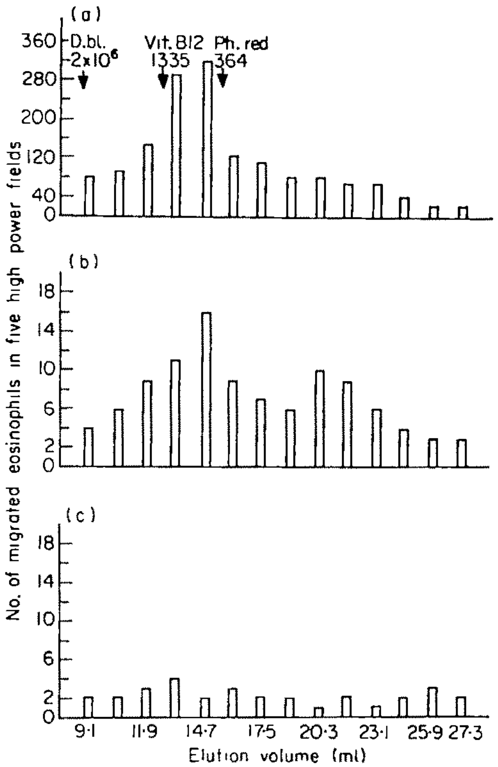

FIGURE 1. Elution pattern of neutrophil derived ECF (a) and of suction blister fluid obtained over involved skin of an atopic eczema patient (b) and the healthy skin of a normal control subject (c). Two hundred microlitres were placed on a 14 × 1·5 cm Sephadex G 25 column. Dextran blue 2000 (D.Bl ), vitamin $B_{12}$ (Vit. $B_{12}$) and phenol red (Ph. red) served as molecular markers.

TABLE 1. Presence of ECF (+), IgE levels and % eosinophils in serum and blister fluid of five patients with bullous pemphigoid

| Patient | Serum (S) Blister (B) | ECF | IgE (u/ml) | Eosinophils (%) |
|---|---|---|---|---|
| 1 | S | + | 300 | 23 |
| | B | + | 80 | 90 |
| 2 | S | + | 70 | 30 |
| | B | + | 90 | 52 |
| 3 | S | + | 1200 | — |
| | B | + | 900 | — |
| 4 | S | + | 150 | 28 |
| | B | + | 110 | 26 |
| 5 | S | + | 510 | 22 |
| | B | + | 120 | 78 |

— not done.

TABLE 2. Summary of occurrence of ECF (+), elevated IgE (> 200 u/ml) and eosinophil (> 5%) levels in blisters of patients with various blistering diseases

| Cause | No. of patients | No. of patients with ECF+ | IgE | Eos |
|---|---|---|---|---|
| Drug reaction (systemic) | 5 | 5 | — | 1 |
| DNCB (contact) | 3 | — | — | — |
| Primula (contact) | 1 | 1 | — | 1 |
| Epidermolysis bullosa | 1 | — | — | — |
| Herpes gestationis | 2 | — | 1 | 2 |

sulphonamides in three cases and salicylates in two. In all instances, ECF was found in the blister fluid, although IgE levels remained within the normal range and local accumulation of eosinophils was found in only one case. One patient with blisters on the dorsa of her hands due to contact with primula had ECF and 19% eosinophils in the blister fluid. In contrast, the patients with DNCB contact blisters, the patient with epidermolysis bullosa and the two patients with herpes gestationis had no measurable ECF in their blisters, even though the latter two patients had increased quantities of eosinophils and the IgE level (300 u/ml) was elevated in one instance.

In a previous publication (Dierksmeier & Czarnetzki, 1978) we reported on the absence of ECF in serum of thirteen patients with atopic eczema despite elevated IgE and eosinophil levels. Since we had noted the lower ECF levels in serum as compared to blister fluid (see above), we induced suction blisters over the skin of patients with this disease in unaffected and eczematous areas. In three patients with atopic dermatitis ECF was demonstrated in the blister fluid obtained from affected, but not from normal, skin in the same patients. Similarly, suction blisters placed over healthy skin of three normal controls contained no ECF.

## DISCUSSION

The findings of this study give, for the first time, evidence for the presence of low molecular weight ECF in a number of blistering dermatological diseases at the involved tissue sites. This emphasizes the importance of ECF for the attraction of eosinophils *in vivo*.

So far, ECF has been demonstrated in only a few selected cases within body fluids. Thus, it was found in the urine of two patients with ECF-secreting lung carcinomas (Goetzl *et al.*, 1978), and in the effluent venous blood (serum) of three patients with cold urticaria after local cold challenge (Soter, Wassermann & Austen, 1976). In addition, Baba *et al.* (1976) succeeded in demonstrating low molecular weight activity in four patients with bullous pemphigoid blister fluid. We were able to confirm the latter findings in our five patients with that disease and, in addition, found ECF in the serum of these patients as well. The failure to do this previously may be due to the lower ECF levels in serum and our more sensitive assay system.

The presence of ECF in blisters of patients with some allergic reactions (Table 2) was an unexpected finding. It has been assumed that the ECF in bullous pemphigoid lesions is mast cell-derived because of elevated IgE levels in this disease and degranulated mast cells at reaction sites (Wintroub *et al.*, 1978). IgE-mediated mechanisms are known to participate in immunological reactions to drugs; yet, phagocytosis of immune complexes at tissue sites by PMN, with subsequent ECF release from these cells, might have occurred as well. At the current state of knowledge, it is not possible to differentiate the ECF from mast cells or PMN by chemical methods and the question of the source of ECF must await final proof.

It is also possible that both mast cell and PMN-derived ECF is generated in allergic reactions, especially since a further eosinophil chemotactic factor, a C5-derived component of complement, has been demonstrated in blisters of patients with bullous pemphigoid (Dia-Perez & Jordon, 1976). Complement activation is known to occur at tissue sites in the presence of immune complexes during drug reactions.

We always noted increased levels of eosinophil chemotactic activity in the void volume of the G 25 column of ECF-positive test substances. The several eosinophil chemotactic factors of larger molecular weight mentioned in the introduction could have contributed to this finding, as well as the larger products from mast cells (Boswell, Austen & Goetzl, 1978) and PMNs (Czarnetzki *et al.*, 1976a). Enhancing factors in serum such as $\alpha_2$ macroglobulin (Czarnetzki & Feldmann, 1978) could, furthermore, have increased the biological activity of these factors in the void volume of the G 25 column. The lack of ECF in some lesions despite the presence of eosinophils (Table 2) speaks for the involvement of other eosinophil chemotactic factors and thus for the activation of several immunological mechanisms.

The presence of ECF in some blister fluids in the absence of local eosinophilia is a puzzling finding. Little is known about the induction of eosinophil development in the bone marrow. While we have shown that ECF can affect the bone marrow (Czarnetzki & Meister, 1978), the quantity of ECF generated locally might not suffice to reach the marrow, especially since ECF is inhibited by serum (König & Czarnetzki, 1978), and disappears from there in a very brief time (Soter *et al.*, 1976). Although eosinophils are absent, ECF can nevertheless be functional since it also attracts PMN (Czarnetzki *et al.*, 1976a), and a very potent effector cell is thus called to the site of inflammation that differs from eosinophils in only a few properties.

The finding of ECF in suction blisters over eczematous skin and its absence from normal skin emphasizes that ECF can be very local in its action. Most likely, it is generated at that same site since serum levels were not detectable in thirteen atopic eczema patients (Dierksmeier & Czarnetski, 1978). Because of known increased histamine and IgE levels in patients with this disease, the mast cell is the most likely source of ECF. Lymphokines might play a role also considering the lymphocyte-enriched local inflammatory infiltrate and the importance that is recently placed on cell-mediated immune mechanisms in the pathogenesis of atopic eczema.

The demonstration of ECF, as reported here, has never been attempted on a larger scale because the methods are rather laborious. With a better chemical characterization of the factors, this task should be easier. Then various mediators involved in the local inflammatory response as well as their source, interaction and fate could be better studied. The pathogenic pattern of disease associated with eosinophilia would thus become more apparent.

## ACKNOWLEDGMENTS

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# Neutrophil dysfunction and repeated infections: influence of levamisole and ascorbic acid

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## SUMMARY

Neutrophil function was studied in several patients with recurrent infections, mainly of the skin. Twelve patients showed impairment of neutrophil functions, either chemotaxis or bacterial killing and phagocytosis.

Levamisole was given in four cases: improvement of neutrophil function and long-lasting clinical remission occurred in three of them, whilst in the fourth the drug was not tolerated.

Ascorbic acid was administered to three other patients, with satisfactory improvement of neutrophil function and long-lasting clinical remission.

Patients with defective neutrophil function frequently display an undue susceptibility to infections, most often involving the skin and the subcutaneous tissue (Quie, 1975; Clark, 1978; Quie & Cates, 1978; Stossel, 1978).

Commonly impaired functions are chemotaxis, bacterial killing and phagocytosis. Defects of adhesiveness or cell deformability have been described only rarely and in general in association with the former dysfunctions (Clark, 1978).

A pathophysiological classification of these disorders is still tentative owing to the heterogeneity of clinical reports and of the laboratory methods which in most cases are fairly recent and still in progress.

Here we report our experience with twelve patients who had repeated skin infections and neutrophil dysfunction, as well as the results of levamisole or ascorbic acid therapy.

## MATERIALS AND METHODS

The patients were investigated on two occasions at least, when free from infections and before therapy had started. Clinical data are shown in Table 1. Studies of cell-mediated immunity revealed only minor impairment in patients A.V., D.O., M.C. and O.L. Neutrophil studies and serological studies were performed as follows.

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TABLE 1. Clinical presentation of twelve patients with defective neutrophil function and recurrent infections

| Patient | Age/sex (years) | Clinical presentation | Function involved |
|---|---|---|---|
| A.V. | 2·5/F | Atopic dermatitis, cutaneous abscesses, sinusitis, otitis media | Chemotaxis |
| D.O. | 11/M | Atopic dermatitis, abscesses, bronchopneumonia | Chemotaxis, killing |
| M.C. | 30/F | Cutaneous abscesses, tonsillitis | Chemotaxis |
| S.A. | 25/M | Folliculitis | Chemotaxis |
| G.I. | 4·5/M | Atopic dermatitis, bronchitis, otitis media, urticaria | Chemotaxis |
| A.S. | 26/F | Folliculitis | Chemotaxis, random migration |
| O.L. | 50/F | Folliculitis | Chemotaxis, phagocytosis, killing |
| M.C. | 8/M | Cutaneous abscesses | Killing |
| V.C. | 9/F | Cutaneous abscesses | Killing, phagocytosis |
| F.C. | 18/F | Folliculitis, otitis media | Chemotaxis |
| M.S. | 3 months/M | Napkin psoriasis, otitis media | Chemotaxis |
| L.C. | 26/M | Furuncles, bronchitis | Chemotaxis |

TABLE 2. Immunological evaluations

| | Neutrophil studies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | *S. aureus* killing | |
| Patient | Random migration (µm) | Chemotaxis (µm) | Spont. NBT (%) | Stim. NBT (%) | Adhesiveness (%) | Phagocytosis (%) | 30 min (% of control) | 60 min |
| A.V. | 25 | 40 | 0 | 13·5 | 73 | 49 | ND | |
| D.O. | 33 | 58 | 1 | 34 | 94 | 48 | 41 | 99 |
| M.C. | 30 | 65 | 0 | 40 | 84 | 82 | 87 | 92 |
| S.A. | 32 | 70 | 2 | 30 | 97 | 90 | 93 | 97 |
| G.I. | 31 | 45 | 1 | 46 | 95 | 61 | ND | |
| A.S. | 10 | 47 | 6 | 40 | 88 | 56 | 97 | 98 |
| O.L. | 32 | 75 | 6 | 29 | 85 | 38 | 36 | 83 |
| M.C. | 34 | 83 | 3 | 53 | 85 | 51 | 66 | 54 |
| V.C. | 33 | 86 | 6 | 48 | 80 | 39 | 34 | 51 |
| F.C. | 21 | 69 | 1 | 32 | 75 | 52 | 89 | 98 |
| M.S. | 23 | 64 | 3 | 34 | ND | ND | ND | |
| L.C. | 17 | 50 | 7 | 35 | 79 | 72 | 127 | 102 |
| Normal range (adults) | 15–35 | 80–120 | 0–7 | 30–60 | 75–95 | 50–80 | 80–120 | |

* Neutrophil myeloperoxidase score.

† Neutrophil alkaline phosphatase score.

‡ Micrometres travelled by normal neutrophils when patient's serum activated with endotoxin was used as chemoattractant.

### *Neutrophil studies*

Chemotaxis and random migration were performed in triplicate according to the method of Wilkinson (1974) using casein 5 mg/ml (Hammarsten, Merck, Darmstadt) as chemotactic stimulus and 3 $\mu$m filters (SSWP02500, Millipore, Milano). The distance ($\mu$m) travelled by cells after 45 min incubation at 37°C was evaluated by the leading front method (Zigmond & Hirsch, 1973) as determined from five randomly chosen fields at ×400 magnification for each filter. Adhesiveness was studied in triplicate by the method of MacGregor, Spagnuolo & Lentnek (1974) using nylon fibres (Fenwal, Travenol, Roma). Results were expressed as the percentage of neutrophils adhering to the nylon column. Spontaneous and endotoxin-stimulated NBT reduction (NBT, BDH Chemicals Ltd, Poole) were performed with the methods of Park, Fikring & Smithwich (1968) and Park & Good (1970) respectively. The percentages of neutrophils containing blue-black formazan deposits were evaluated by counting at least 200 consecutive cells. Phagocytosis was studied by the method of Chrétien & Garagusi (1972) using polystyrene particles (0·81 $\mu$m diameter, Difco, Detroit). Results were expressed as the percentage of neutrophils containing latex particles. Bactericidal power against *Staphylococcus aureus* (*S. aureus* A8 kindly supplied by Dr P.E.Varaldo, Department of Microbiology, University of Genoa) was assessed in triplicate using the method reported by Wilkinson (1977) with a simultaneous normal control. Briefly, neutrophils were allowed to phagocytize bacteria (bacteria to neutrophil ratio of 10:1) by incubation (20 min at 37°C in a water-bath shaker) in Hanks solution containing a 10% final concentration of pooled normal serum. After centrifugation (5 min, 400 *g*) the sedimented cells were resuspended in Hanks plus 10% fetal calf serum, penicillin (100 u/ml) and streptomycin (100 $\mu$g/ml) and the mixtures were reincubated. At 0, 30 and 60 min, samples were removed and centrifuged. The cellular pellets were washed twice in Hanks solution and resuspended in distilled water. Serial dilutions were made and the number of viable bacteria was determined by the pour plate technique. The results were expressed as percentages of surviving bacteria at each time as compared to simultaneous control samples. Neutrophil alkaline phosphatase and peroxidase activity was evaluated

TABLE 2. Immunological evaluations (*continued*)

| Neutrophil studies | | Serological studies | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MPO* | AP† | IgG (mg/dl) | IgA (mg/dl) | IgM (mg/dl) | IgE ($\mu$g/ml) | C (mg/dl) | C4 (mg/dl) | CF‡ |
| 185 | 87 | 700 | 35 | 120 | 1800 | 62 | 27 | 96 |
| 198 | 43 | 800 | 112 | 165 | 800 | 120 | 30 | 90 |
| 190 | 48 | 1250 | 320 | 165 | 76 | 100 | 35 | 81 |
| 196 | 41 | 1400 | 315 | 150 | 200 | 115 | 37 | 100 |
| 180 | 27 | 1080 | 160 | 105 | 300 | 120 | 40 | 85 |
| 199 | 93 | 1220 | 305 | 174 | 20 | 75 | 30 | 99 |
| 190 | 35 | 2000 | 650 | 240 | 800 | 115 | 31 | 80 |
| 200 | 45 | 990 | 185 | 87 | 90 | 80 | 40 | 81 |
| 180 | 30 | 970 | 172 | 92 | 120 | 62 | 35 | 84 |
| 200 | 21 | 1200 | 220 | 155 | 220 | 120 | 37 | 96 |
| 195 | 42 | 700 | 56 | 68 | 20 | 64 | 28 | ND |
| 187 | 23 | 1020 | 234 | 90 | 190 | 105 | 32 | 84 |
| 175–225 | 20–100 | 800–1500 | 120–400 | 80–200 | 20–300 | 55–120 | 20–40 | 80–120 |

by cytochemical methods (Dacie & Lewis, 1975). At least 200 consecutive cells were scored according to a scale ranging from 0 to + + + + for alkaline phosphatase and from 0 to + + + for peroxidase, and the results were expressed as the mean score per 100 cells.

### *Serological studies*

Serum concentrations of IgG, IgA, IgM, C3 and C4 components of complement were determined by radial immunodiffusion using Behringwerke immunoplates (Mancini, Carbonara & Heremans, 1965). IgE concentration was measured by the Phadebas IgE test (Pharmacia, Uppsala).

Generation of complement-derived chemotactic factors was evaluated by the method of Gallin, Clark & Frank (1975) using 100 μg/ml endotoxin (*E. coli*, 0111:B4, Difco, Detroit).

Cell-directed inhibitory activity of chemotaxis, phagocytosis and bacterial killing were assayed by incubating normal neutrophils (30 min, 37°C) in patients' serum before the function tests were performed.

### *Therapy studies*

In four patients with defective neutrophil chemotaxis (A.V., D.O., M.C., G.I.) levamisole (2·5 mg/kg, 3 days/week) was given. Three patients with predominant defective bacterial killing (O.L., M.C., V.C.) were treated with ascorbic acid (1–2 g/day).

In these patients, neutrophil function was repeatedly evaluated during treatment.

## RESULTS

As seen in Table 2, ten of our patients had defective neutrophil chemotaxis. In six cases, chemotaxis was the only neutrophil function affected. Four patients had defective *Staphylococcus aureus* killing, which, in one case, was the sole function affected. Occasionally abnormality of random migration, NBT reduction and phagocytosis were found in association with defects of chemotaxis and killing. Some patients had abnormal serum immunoglobulin levels (hyper IgE and hyper IgA) but the impairment of neutrophil function was never due to cell directed inhibitors nor to chemotactic-factor-generation abnormalities. Only in O.L., who had an intrinsic bactericidal activity deficiency, was a slight cell-directed chemotactic inhibitory activity found.

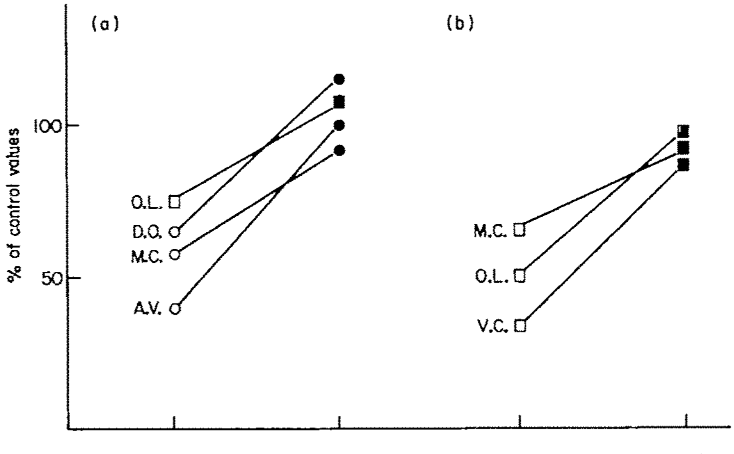

FIGURE 1. Neutrophil chemotaxis (a) and *Staphylococcus aureus* killing (b) before (open symbols) and after (solid symbols) therapy with levamisole (●) or ascorbic acid (■). Results are expressed as per cent of control values.

Levamisole therapy was effective in restoring neutrophil chemotaxis. Figure 1 shows the improvement of neutrophil function after therapy lasting from 1 to 3 weeks. In one case (M.C.) the drug was not tolerated for more than 2 weeks. In the other three patients, who received 4 weekly courses and then additional courses at intervals to reach 8 (A.V.), 16 (D.O.) and 20 (G.I.) during the first year, clinical improvement was also seen, with progressive clearing of infections and complete lack of relapse. In two patients (A.V. and G.I.) additional therapy was given during the second year of follow-up, when they had already been free of infection for several months.

Ascorbic acid therapy was associated with improvement of neutrophil function after a few weeks (3–6), as seen in Fig. 1. This treatment, which has been continued till now, has also given clinical results comparable to those obtained with levamisole.

## DISCUSSION

A survey of the literature as well as our experience suggests that the main dermatological manifestations presented by patients with neutrophil dysfunction are recurrent pyoderma and atopic-like dermatitis; less frequently these patients may show urticaria, mucocutaneous candidiasis, trichophytosis, ichthyosis or incontinentia pigmenti; oculocutaneous albinism is a peculiar feature of the Chediak-Higashi syndrome (Quie, 1975; Dahl, Greene & Quie, 1976; Clark, 1978; Rabson, Anderson & Glover, 1978).

Recurrent pyoderma may present in various ways; however, dermatological advice is usually sought for deep-seated abscesses (Dahl *et al.*, 1976; Rebora *et al.*, 1978). These may be located in various areas of the skin and may recur with extraordinary frequency; one of our cases (D.O.) had forty-three abscesses in 2 years. The onset may be very early, more frequently between the second and tenth year, and rarely in adult life.

Abscesses may be associated with, or alternate with, minor furuncles and superficial folliculitis, most often on the face and on the buttocks and which may sometimes mimic acne.

'Cold' abscesses have been described in a few cases, without the usual inflammation; they are characteristic of so-called Job's syndrome (Davis, Schaller & Wedgwood, 1966) affecting females with red hair and hyperextensible joints. This syndrome is exceedingly rare and considered by some authors as a subgroup of the hyper-IgE syndrome; we do not have any direct experience of it.

Unlike with other immunodeficiencies, viral infections are usually absent and septicaemia or involvement of the central nervous system have only rarely been observed. *Streptococcus pneumoniae*, *Haemophilus influenzae* and Group-A streptococci are frequently found in patients with hypogammaglobulinaemia, while in neutrophil dysfunctions, *Staphylococcus aureus* predominates (Quie & Cates, 1978). Candida infection, which has been related to concomitant T-lymphocyte dysfunction, is also found in a minority of patients with neutrophil dysfunction.

In our experience the clinical features of pyoderma do not significantly differ with the type of functional defect.

A chronic itching dermatitis is the second important skin manifestation in these patients and has most of the features of atopic dermatitis. As pointed out by Buckley, Wray & Belmaker (1972), some of its aspects are atypical and the classical features of atopic dermatitis are so intermingled with those of minor infections that a clear description cannot be made (Stanley *et al.*, 1978).

Atopic patients have been recognized to be heavy carriers of *Staphylococcus aureus* (Leyden, Marples & Kligman, 1974; Aly, Maribach & Shinefield, 1978) and Rogge & Hanifin (1976) went so far as to say that in atopics the more severe the dermatitis the higher the prevalence of infection and the more severe the abnormality of leukocyte function. In the present series this was not the case. In fact, impaired neutrophil chemotaxis is not constantly associated with atopic dermatitis and is a feature only of the hyper-IgE syndrome (Dahl, Cates & Quie, 1978). This eruption is usually absent from other syndromes with defects of leukocyte function. Our cases O.L., who had a killing deficiency, and F.C., who had a defective chemotaxis, did not show any eczema.

Urticaria has been described in a minority of such patients. We found it only in the case G.I., who had a family history of asthma. Candidiasis, in its chronic mucocutaneous form and trichophytosis have been found in only a few cases; one of ours (A.V.) had vaginal candidiasis.

Nail dystrophy has been described in Job's syndrome (Pincus *et al.*, 1975), looking much like the nail dystrophy observed in chronic mucocutaneous candidiasis (Quie & Cates, 1978); we have not found it in our patients.

The association with ichthyosis and incontinentia pigmenti described in two cases (Hill *et al.*, 1974; Dahl *et al.*, 1975) is probably fortuitous as it was with the psoriasis of our case (M.S.).

Also, the 'coarse facial features' originally noted by Buckley *et al.* (1972) and the 'broad nasal bridge' claimed by Quie & Cates (1978) in Job's syndrome are not a constant finding, in our experience at least.

As far as their classification is concerned, our cases can be defined as due to intrinsic defects; no humoral inhibitors have been found except a slight cell-directed inhibitory activity to chemotaxis in a single case (O.L.). Cases A.V. and D.O. can be included in the hyper-IgE syndrome of Buckley, patient D.O. being the first example associated with a defect of bactericidal power. Case G.I. shows a transient neutrophil chemotactic defect like that recently described by Hill *et al.* (1976) in four patients. Case A.S. is an example of the very rare lazy leukocyte syndrome (Miller, Oski & Harris, 1971). The other patients fall into that group of heterogeneous cases which comprises the large majority of reports. Case O.L. closely recalls the patient described by Tan *et al.* (1974).

In most cases the cause of the intrinsic functional defect is unknown. In the hyper-IgE syndrome an IgE-dependent inhibition due to histamine has been suggested (Hill & Quie, 1974). However, the plasma level of IgE does not parallel the severity of the chemotactic defect (Quie & Cates, 1978) and till now an increase of histamine in blood or in leukocytes of these patients has not been proven.

In some patients with the hyper-IgE syndrome the chemotactic defect has been elicited *in vitro* only after contact with particular antigens (Rubin, Griffiths & Hill, 1978); this finding may provide a good explanation for the transient defect, such as in our case G.I.

The disorder of random migration, which is concomitant with the chemotactic defect in the lazy leukocyte syndrome, is probably related to severe impairment of the basic locomotor mechanisms of the cell (Malech, Root & Gallin, 1977).

As for killing defects, a faulty oxidative mechanism has been elucidated, at least in part, in chronic granulomatous disease, the Chediak Higashi syndrome and myeloperoxidase deficiency (Babior, 1978). Our cases, however, cannot be ascribed to those syndromes, since they showed a normal or only slightly impaired NBT reduction (case A.V.), normal myeloperoxidase content and were lacking the typical features of the Chediak-Higashi patients.

The pharmacological correction of neutrophil functional disorders is at present under investigation. The most promising drugs are levamisole, ascorbic acid and $H_2$-blocking agents (Clark, 1978). With levamisole the clinical results have been somewhat contradictory (De Cree *et al.*, 1978; Rabson *et al.*, 1978). We have found good and long-lasting results in cases A.V., D.O. and G.I. whilst in case M.C. the drug was not tolerated. As for ascorbic acid, we have found excellent results in cases O.L., M.C. and V.C. since they have been free from infections for 1 year. Though we cannot be absolutely sure that the clinical improvement of these patients was really due to the therapy, this is suggested by the improvement of neutrophil function after therapy and by the fact that the patients had never experienced such long periods of well-being since the first appearance of the disease. We wish to stress in particular that the laboratory and clinical results obtained with ascorbic acid in our patients and the safeness of this drug strongly suggest its use for the prevention and treatment of the recurrent infections in patients with defective chemotaxis and/or bacterial killing.

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# Serum immunoglobulin values in patients with scabies

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## SUMMARY

Serum total IgE levels were found to be increased in thirty-nine of ninety-four patients with scabies investigated at the time of clinical presentation. One hundred and fifty-four patients were found to have a significantly lower serum concentration of IgA during infection than when measured 6 weeks and 9 months following successful treatment ($P<0{\cdot}05$). The serum concentration of IgA at 6 weeks and 9 months after treatment did not differ significantly. The serum concentration of IgG during infection was significantly higher than 6 weeks later ($P<0{\cdot}05$), and than 9 months later ($P<0{\cdot}001$). The 6 weeks levels were significantly higher than the results at 9 months ($P<0{\cdot}01$). The serum concentration of IgM during infection was higher than 6 weeks later ($P<0{\cdot}1$). The 6 weeks levels were lower than those at 9 months ($P<0{\cdot}1$), whereas there was no difference between the pretreatment and 9 months levels. No differences were noted in the concentration of C3 and C4. Antinuclear antibodies (ANA) and rheumatoid factor (RF) were not found in any of the patients. In thirty-two patients with lichen planus no changes in immunoglobulins or complement were observed.

This suggests that scabies infection may be associated with a humoral immunological response against the scabies infection.

The spreading of human scabies, and fluctuations in its incidence, may be due to a number of different factors. It has been suggested that immunological conditions play an important role, but so far neither an antigen in scabies mite nor antibodies against it have been demonstrated. It is therefore not certain whether the immunological response to scabies infection is cell-mediated or humoral (Falk, 1980).

This study was designed to investigate the serum immunoglobulin values during and after scabies infection.

## MATERIALS AND METHODS

### *Patients*

This study involved 154 patients with scabies (seventy-nine females, seventy-five males) aged from 11 to 76 years (average 25·9 years). In all patients diagnosis was confirmed by the presence of one or more living mites. All patients under 11 years of age were excluded, to avoid unnecessary blood tests in young children. None of the patients investigated had earlier been infected with scabies and the pruritus or skin rash varied in duration from 1 week to 12 months. All visible adult female mites were

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manually removed. In each patient we noted the number of mites, and the extent, intensity and duration of the rash, and compared these with the serum immunoglobulin values.

*Sera*

Blood samples were drawn from each patient at the time of clinical presentation and 6 weeks and 9 months later; after clotting for 2 h at room temperature the sera were separated and stored at −20°C until the tests were performed.

*Serum IgE levels*

IgE was determined using the Phadebas PRIST test (Pharmacia AB, Uppsala, Sweden). The upper limits of normal IgE values are, at 10 years of age, 182 u/ml; at 14 years of age, 195 u/ml; and in adults 122 u/ml (Nyco, Oslo, Norway). In an unselected group of ninety-four of the 154 patients, IgE was determined at the time of clinical presentation. The tests were not repeated after 6 weeks and 9 months.

*Serum immunoglobulin A, G, M, C3 and C4*

The determination of the IgA, IgG, IgM, C3 and C4 concentrations in the sera of the 154 patients was made by single radial immunodiffusion. All the patients returned to the clinic after 6 weeks and sixty-five (thirty-four females, thirty-one males) returned a third time to the clinic after an average of 9 months, when the same parameters were measured (Table 1).

*ANA and RF*

The sera at the time of the clinical presentations were tested for antinuclear factor (ANF), Rose-Waaler test and Latex test.

In a control group of thirty-two patients with lichen planus similar studies of immunoglobulins and complement were made at the outbreak of the disease and 6 weeks after treatment.

Statistical significance was calculated using three methods: sign test, Wilcoxon rank order test and Student's '$t$'-test.

## RESULTS

Fewer than ten mites were found in 119 patients (77%), ten to fifty mites in twenty-six patients (17%) and more than fifty mites in nine patients (6%). One of these nine patients had as many as 250 mites, the majority being found on the fingers, palms and on the inside of the wrists. All the patients responded to treatment.

The results of the serum immunoglobulin values are given in Table 1 and Fig. 1.

Twelve of the patients were between the ages of 11 and 14 years, and had IgE levels of <10–550 u/ml. Four of these twelve patients had elevated IgE levels of 270, 350, 450 and 550 u/ml. Eighty-two adults had IgE levels of <10>1000 u/ml. Thirty-five of the eighty-two adult patients had IgE levels >122 u/ml. Twenty had IgE levels of 125–300 u/ml, five of >300–500 u/ml, four of >500–1000 u/ml and six of >1000 u/ml.

The serum concentration of IgA during scabies infection was found to be significantly lower than 6 weeks after successful treatment ($P<0\cdot05$). The serum concentration of IgA during scabies infection was also found to be significantly lower than 9 months later ($P<0\cdot05$), whereas there was no significant difference in the serum concentration of IgA at 6 weeks and 9 months after treatment.

The serum concentration of IgG during infection was significantly higher than 6 weeks later

TABLE 1. Serum immunoglobulin A, G and M levels in 154 patients with scabies

| | During infection | | 6 weeks later | | 9 months after treatment |
|---|---|---|---|---|---|
| | ↓ | $P < 0.05$ | ↑ | | |
| IgA | ↓ | | | $P < 0{\cdot}05$ | ↑ |
| | ↑ | $P < 0{\cdot}05$ | ↓ | | |
| IgG | ↑ | | | $P < 0{\cdot}001$ | ↓ |
| | | | ↑ | $P < 0{\cdot}01$ | ↓ |
| | ↑ | $P < 0{\cdot}1$ | ↓ | | |
| IgM | ← | ——— | ——— | ——— | → |
| | | | ↓ | $P < 0{\cdot}1$ | ↑ |

C3 and C4 did not differ significantly. ANA and RF were not found.

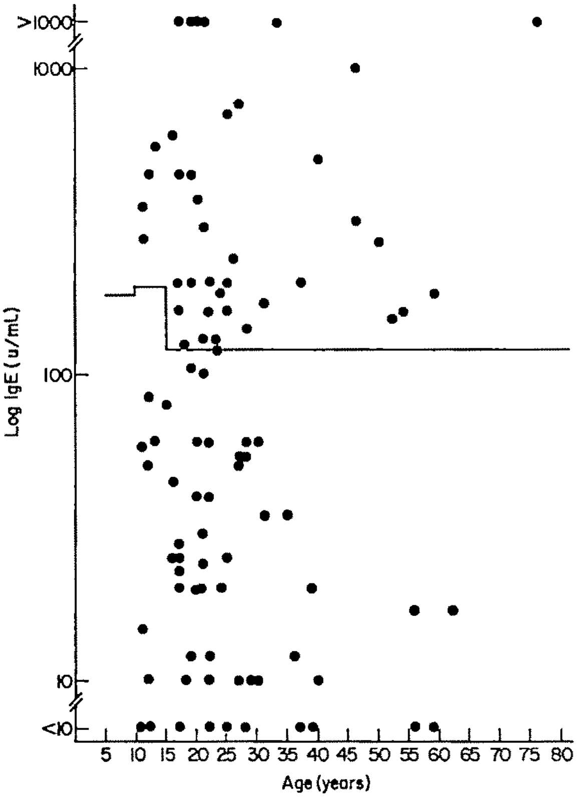

FIGURE 1. Range of IgE values in ninety-four patients with scabies. Solid line shows upper limit of normal IgE values.

($P < 0{\cdot}05$) and than 9 months later ($P < 0{\cdot}001$). The 6 weeks levels were significantly higher than the results at 9 months ($P < 0{\cdot}01$).

The serum concentration of IgM during infection was higher than 6 weeks later ($P < 0{\cdot}1$). The 6 weeks levels were lower than the results at 9 months ($P < 0{\cdot}1$), whereas there was no difference between the pretreatment and 9 months levels.

No differences were seen in the serum concentration of immunoglobulins and complement due to either age or sex, and no correlation to the duration of the disease could be observed. Although there was no definite correlation between serum immunoglobulin values and the actual number of mites or the duration or intensity of rash, the number of mites did to a certain extent seem to be dependent upon the degree of sensitization in the host. In cases with high numbers of mites there was little local inflammation around the burrows and mites, and few scratch marks.

The ANF, Rose-Waaler and Latex test were in all cases negative. There was no difference in the serum concentrations of C3 and C4 prior to or following treatment.

In the control group of thirty-two patients with lichen planus no changes in immunoglobulins and complement were seen at the outbreak of the disease and in remission.

## DISCUSSION

All the patients in the present study were successfully treated and this can probably be attributed to our removing all the visible adult female mites manually prior to treatment.

We found increased serum IgE levels in thirty-nine of ninety-four patients with scabies at the time of clinical presentations. It is often said that increased IgE levels indicate either allergic disease or intestinal or systemic parasitism. In the world literature there are no available controlled investigations as to whether there are differences in that respect between external and internal parasitism. Hancock & Ward (1974) found all IgE levels except one within normal limits in 100 patients with scabies. The number of increased IgE levels in this study is surprisingly high and is in contrast to the study by Hancock & Ward (1974). A possible explanation for the increased IgE values could be that these patients had atopic illness. However, an increased serum concentration of IgE need not indicate atopy. In most of these patients there was no obvious history of atopy, but a comprehensive and systematic medical history supplemented with skin tests would be necessary to clarify this problem. The most likely explanation is that the scabies infection is at least partly responsible for the increased IgE values. Unfortunately the IgE investigations were not repeated, but a further study is in progress in which the IgE levels will be followed.

The values of IgA during scabies infection were significantly lower than 6 weeks and 9 months after successful treatment ($P<0{\cdot}05$), whereas there was no significant difference between the values of IgA at 6 weeks and 9 months after treatment. Hancock & Ward (1974) found IgA levels that were significantly lower than normal. The mean values for IgG and IgM were significantly higher than the normal mean values. A dermatosis control group showed essentially normal values for IgM and a mean IgG and IgA value which was significantly higher than the mean. They suggest a possible relationship between increased IgG/IgM and systemic infections and scabies sensitization, and also that the low serum IgA may correlate with low secretory IgA in skin secretions predisposing to scabies infestation. Since the decreased IgA level during scabies infection returns to normal, the most likely explanation must be that processes related to the infection cause the decrease. This is in contrast to the study by Hancock & Ward (1974) where the IgA level was measured only during the infection, and where it was inferred that the decreased serum IgA concentration existed prior to the infection and predisposed to scabies infestation. The primary and main site of IgA synthesis in the body is suggested to be the plasma cells of the intestinal mucosa (mainly the lamina propria). Nevertheless, a considerable proportion of IgA seems to be destined for local functions. It has been suggested that IgA is involved in humoral immunity since it can be demonstrated in relatively large quantities in the external secretions, including sweat, after local stimulation (van der Meer, 1973). Histological examination of scabies skin lesions often reveals a pleomorphic perivascular cellular infiltrate which is frequently most pronounced around the sweat glands (Thomson *et al.*, 1974; Hejazi & Mehregan, 1975). If IgA is not synthesized locally in the skin, a possible explanation for the decreased serum IgA levels during scabies infection could be that the infection causes a transfer of IgA from the serum to the scabies skin lesions.

In the present study a significantly higher serum concentration of IgG was found during infection than 6 weeks later ($P<0{\cdot}05$), and than 9 months after treatment ($P<0{\cdot}001$), the values for IgG also being significantly higher at 6 weeks than at 9 months after treatment ($P<0{\cdot}01$). The IgG values seem to decrease gradually after treatment. This suggests a possible relationship between increased IgG, systemic infection and scabies sensitization. The IgM values during infection were higher than 6 weeks later ($P<0{\cdot}1$). The 6 weeks levels were lower than the results at 9 months ($P<0{\cdot}1$), whereas there was no difference between the pretreatment and 9 months levels. These moderate changes are difficult to explain, and may be of no importance.

The present observations give no indication as to whether the observed changes in immunoglobulin levels represent a non-specific reaction or a specific immunological reaction against the scabies mite. However, in a control group of patients with lichen planus no significant changes in immunoglobulins and complement were measured.

These observations suggest that scabies infection may be associated with a humoral immunological response against the scabies infection. Attempts at demonstrating a scabies mite antigen and antibodies against it are continuing.

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# A technique for studying the structural detail of isolated human corneocytes

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## SUMMARY

A technique for the isolation of human stratum corneum cells from any depth within the horny layer is described. Examination of the cells by light, scanning and transmission electron microscope permits a detailed morphological profile of the plasma membrane to be constructed. It is suggested that the technique will be of particular value in determining the effect of chemical agents on the cellular components of the horny layer.

The stratum corneum protects the vulnerable soft inner tissues from injurious external stimuli and acts as a barrier permitting a controlled interchange of materials with the environment. Despite its importance comparatively little is known of its functional anatomy. Detailed ultrastructural studies of whole stratum corneum from biopsies allow close inspection of the interior of corneocytes but, because of the vertical plane of section, a limited view of the plasma membrane is all that is obtained. In addition, the almost detached state of the outer layers of the stratum corneum makes loss of this zone virtually certain with routine preparation and sectioning techniques. These technical stumbling blocks have effectively prevented proper examination of the stratum corneum exposed to the environment and about to desquamate. This report describes our efforts to overcome the above difficulties and preliminary results using a simple technique.

## METHODS

### *Obtaining corneocytes for examination*

Corneocytes were obtained from the flexor aspect of the forearms of normal human volunteers using the scrub apparatus described by Nicholls & Marks (1977). In this technique the horny cells are collected in 0·06 M phosphate buffer (pH 7·2) containing 0·1% Triton X 100 after agitating the surface of the skin in a controlled and standardized manner with a rotating perspex blade. The corneocytes from, in most instances, five scrubbed sites were pooled to obtain sufficient material for all the procedures undertaken. The pooled suspension of corneocytes was concentrated by centrifugation at 700 *g* and the supernatant decanted off. Approximately two-thirds of the remaining slurry was then

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transferred to an Eppendorf tube and prepared for transmission electron microscopy (TEM) and the remainder was used for light and scanning electron microscopy.

### *Transmission electron microscopy*

The corneocytes were pelleted by centrifugation at 14,000 *g* for 5 min. The supernatant was removed and 1 ml of 0·1 M cacodylate buffered 4% glutaraldehyde (pH 7·4) added. Fixation was allowed to proceed for 1 h. The pellet was then briefly washed with two changes of cacodylate buffer and post fixed in 2% aqueous osmium tetroxide for 30 min. The fixed corneocyte pellet was dehydrated in graded alcohols and embedded in Araldite in the Eppendorf tube. After suitable trimming the resulting block was sectioned on a Cambridge Huxley ultramicrotome. Silver-gold sections were mounted on copper grids and contrasted with saturated alcoholic uranyl acetate for 2 min followed by Reynolds' lead citrate (Reynolds, 1963) for 7 min and finally viewed in an A.E.I. Corinth 275 electron microscope.

### *Scanning electron microscopy and differential phase interference light microscopy*

*Method A.* The residue of the suspension was aldehyde fixed as above, washed and dehydrated in graded alcohol, smeared onto glass microscope slides and then air dried. Representative slides were cut to size and stuck to scanning electron microscope (SEM) stubs before coating with gold in a Edwards 150 sputter coating unit. These were viewed in a Cambridge Stereoscan II or an ISI Super III-A SEM. Other slides were viewed after either mounting in buffered glycerine jelly or directly by differential phase microscopy in a Nikon Apophot microscope (LM).

*Method B.* Cells were also dried by the critical point method to minimize any drying artefacts. For this purpose a chamber was devised and built to hold the cells during processing and to facilitate the critical point drying process. The chamber shown in Fig. 1 consisted of an aluminium cylinder with an internal screw thread. A glass fibre filter (type A, Gelman Instrument Co.) or an 8 μm pore size polycarbonate membrane (Nucleopore corporation) was cut to size using a punch and placed at the base of the cylinder, C, and the spacer, S, positioned above it. The cell suspension was then introduced and a second filter placed between the spacer and the final component B, a cylindrical brass locking screw, which held the whole apparatus in place. This system allowed free flow of fluids through the chamber. When this technique was used an aliquot of the corneocyte suspension was first removed and examined by LM as in Method A above. The remainder was processed in the chamber by fixation with glutaraldehyde and washed as above with subsequent acetone dehydration and critical point drying via $CO_2$ in a Polaron E3000 Critical Point Drying Apparatus. Both filters were mounted on a stub, coated and viewed on an SEM as above.

## RESULTS

### *Efficacy of the technique*

There was some loss of cells, as could be expected, from pipetting the suspension and removal of excess buffer, and between each stage of preparation for SEM by Method A. This was not experienced using Method B, and centrifugation at 14000 *g* ensured that very few cells were lost from the TEM specimen. However, with Method B care had to be taken to use the optimum amount of suspension, too much resulting in large clumps of corneocytes on the filter, thus obscuring some detail. Use of the polycarbonate membranes gave better results than the glass fibre filters, though their transparency made them more difficult to use.

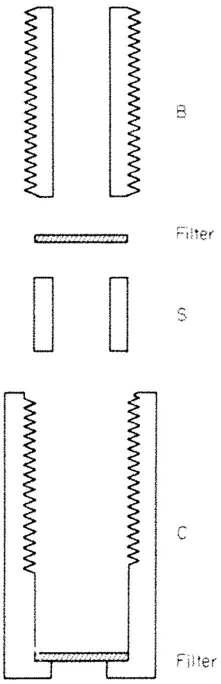

FIGURE 1. Apparatus for containing corneocytes during fixation, dehydration and critical point drying. C = cylinder; S = spacer; B = brass locking screw. See text for description.

### *Structural observations*

Observations at low magnification in both LM and SEM revealed similar information and the following description derives from both techniques (Fig. 2). Corneocytes had a generally polygonal shape but were somewhat irregular. There was no evidence that the preparative technique had damaged the cells and no corneocyte fragments or ruptured cells were seen. The cells were characterized by several marked linear folds in the surface. These sometimes traversed the whole cell surface but often only reached halfway across. They were not all arranged in the same plane and it was not uncommon for the folds to criss-cross the cell at 90° to each other. Apart from these major folds there were also many more subtle undulations of the surface. At higher magnification, small round pits could be seen in many samples but this feature varied greatly from cell to cell (Fig. 3). Cells prepared by Method A often had a very flattened appearance (Fig. 2) when compared to those which had been critical point dried. The latter were often 'curled up' revealing their irregular edges (Fig. 4). This and a reduced number of large folds were the only observed differences between cells prepared by the two methods. On the surface of some cells observed unfixed but air dried directly from the scrub fluid suspension there appeared to be a fine reticular network (Fig. 5). This network did not always end at the cell margin and was quite distinct from the undulations and folds in the surface. There were sometimes rounded expansions in the reticulum. The individual fibrils comprising the reticulum were approximately 0·2–1 $\mu$m in diameter and branched irregularly and at bizarre angles. It did not resemble any type of microbial reticulum.

The typical appearance of the sectioned corneocyte pellet in TEM was as seen in Fig. 6 which shows portions of several different corneocytes. The orientation of the cells appears to be haphazard

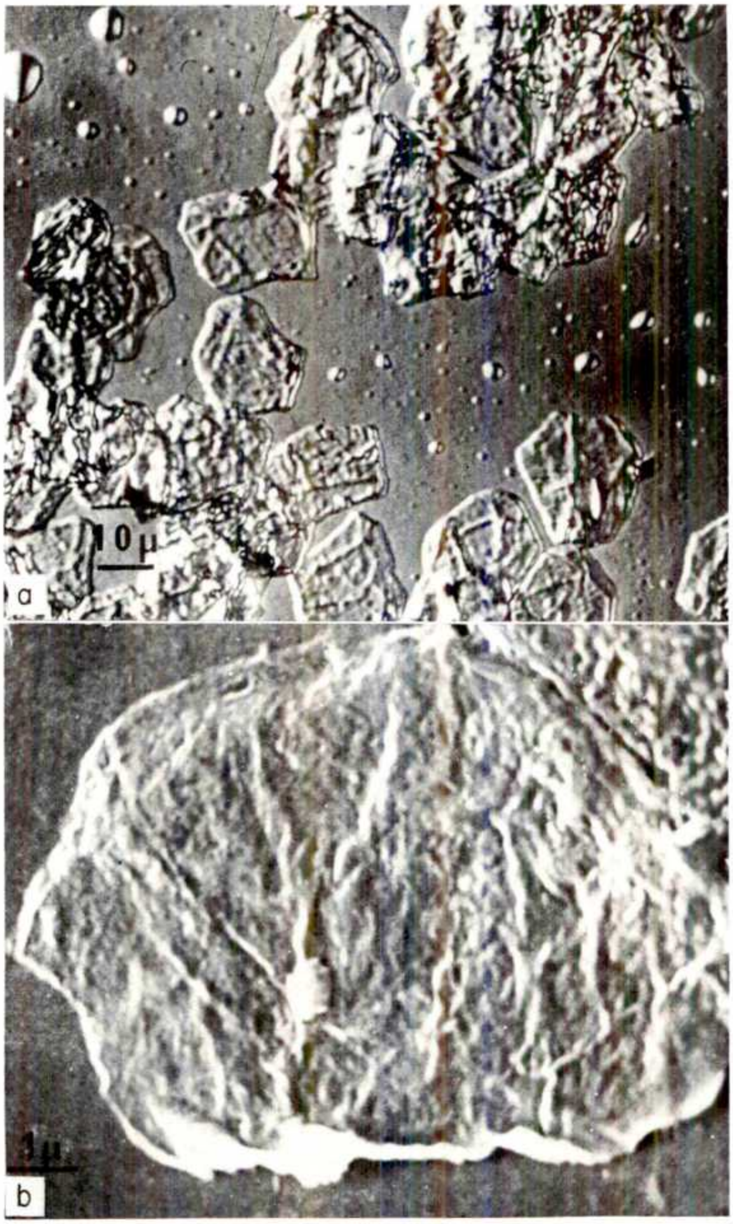

FIGURE 2. (a) Photomicrograph taken by differential phase interference microscopy of corneocytes released by the scrub technique. (b) Scanning electron micrograph of a fixed, dehydrated air dried corneocyte. Both show the major features of isolated corneocytes, conspicuous folds and a generally undulating surface.

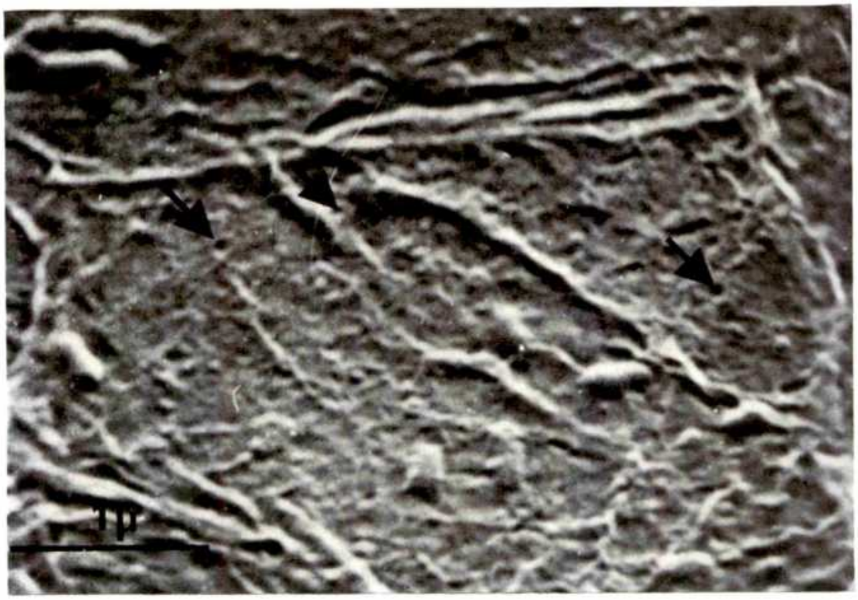

FIGURE 3. Scanning electron micrograph showing the corneocyte surface in greater detail. Small pits (arrows) can be seen as a separate entity from the undulating, folded corneocyte membrane.

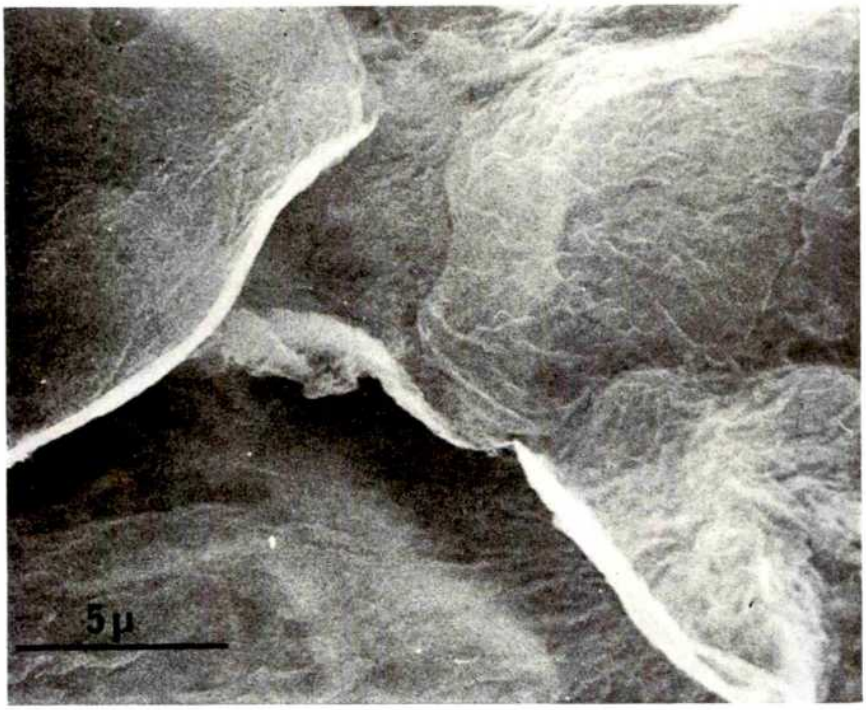

FIGURE 4. Scanning electron micrograph of critical point dried corneocytes. Fewer major folds are visible but the subtle undulations are still present. The narrow edge of the corneocyte can often be seen in corneocytes prepared by this method.

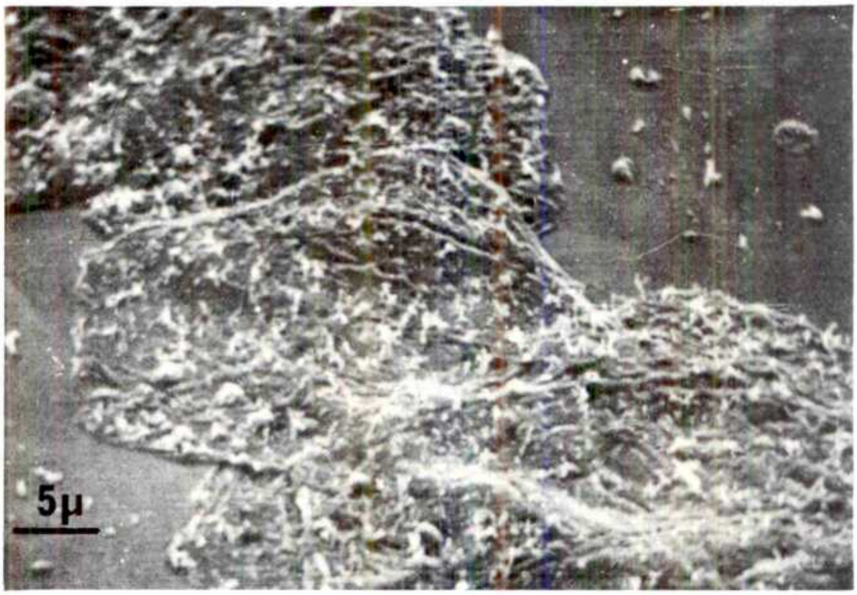

FIGURE 5. Scanning electron micrograph of unfixed air dried corneocytes showing a fine reticular network across their surface.

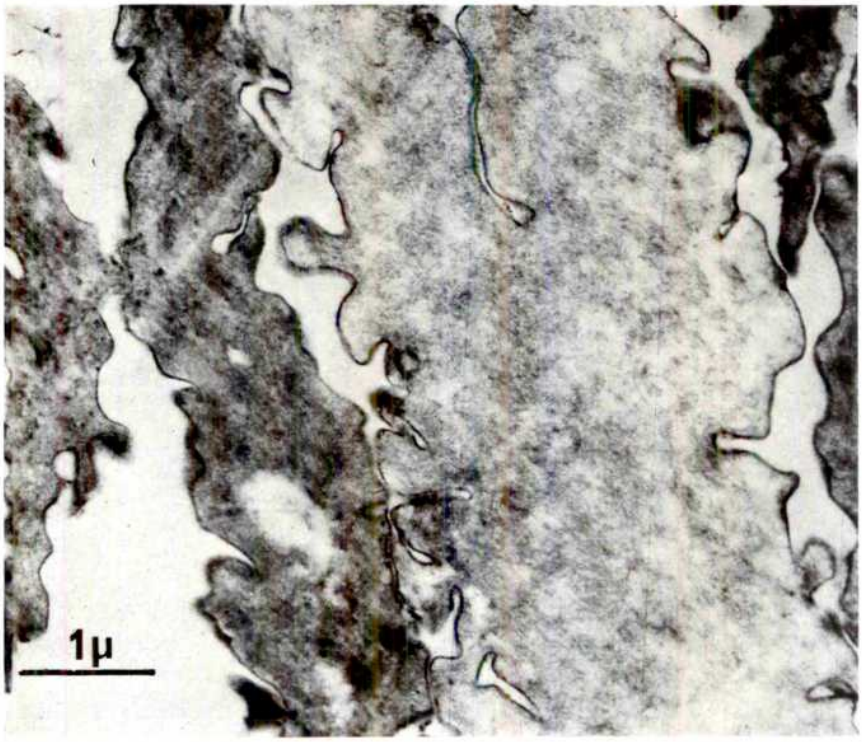

FIGURE 6. Section through a corneocyte pellet. This section passes through different planes of each cell, giving each a different appearance with respect of fibrillar density and intensity of membrane invaginations.

in the pellet and this resulted in a variety of appearances of the cell membranes. The trilaminate plasma membrane proper was rarely seen. There was a very low contrast outer lamina seen in some sections, but the inner lamina was contiguous with the thickened electron dense internal margin of the cell. The width and electron density of this component varied with the plane of section. Cells also varied in their internal structure, some being quite obviously and predominantly fibrillar whilst others had more lightly stained fibrils whose density within the cell appeared to vary. In general, however, the fibrillar pattern conformed to that seen in routinely sectioned biopsy material. An exception to this was the occasional observation of cells within which the fibrillar density varied drastically or was barely discernible. Large dense melanin granules were also distributed among the fibres. The surface foldings seen in the SEM were mirrored by the variety of indentations seen in section and in almost totally horizontal sections of the cell 'holes' were seen. Where these indentations occurred the membrane became much less distinct, wider and less dense, and fibrils could be seen in very close contact with the membrane. This appearance also occurred around holes, occasionally at points where two apparently different cells fused and indeed at any place where the membrane was sectioned obliquely. Apart from these observations, the cell membranes sometimes had closely associated extracellular particulate matter (Fig. 7).

By stripping the skin with adhesive tape serially ten and twenty times and repeating the scrubbing, we obtained cells from the deeper strata of the normal stratum corneum. The corneocytes so collected were generally more substantial in appearance (Fig. 8) and often had a more irregular craggy surface. Critical point dried corneocytes from the deeper strata of the stratum corneum also showed the same tendency, occasionally exhibiting a microvillous form. These exaggerated contours were also occasionally seen in the TEM, but the most striking observation in section was that of intact clumps of

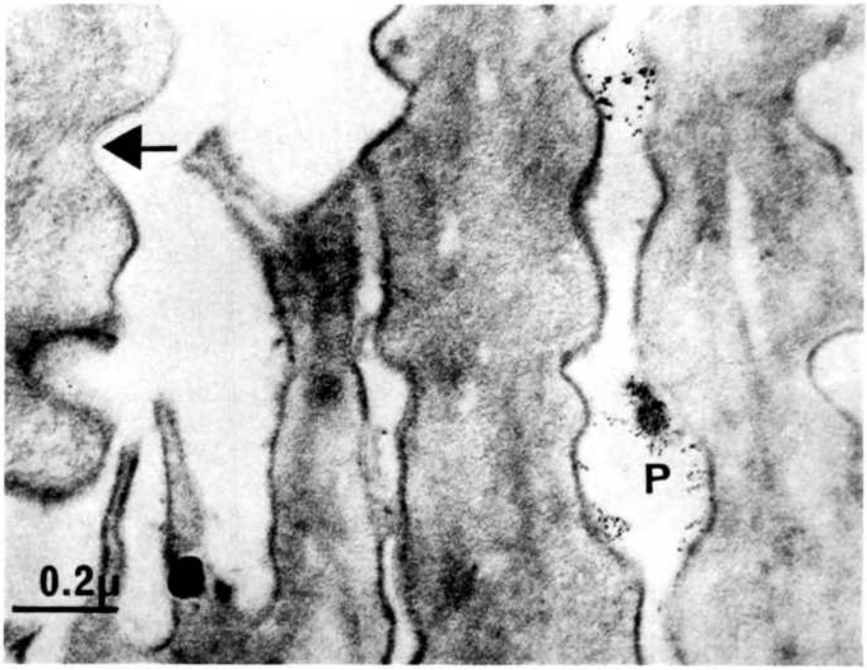

FIGURE 7. Section through several corneocyte regions. The outer plasma membrane proper is just discernible in various places (arrow). The internal margin is of more or less constant thickness. Particulate matter (P) can be seen extracellularly.

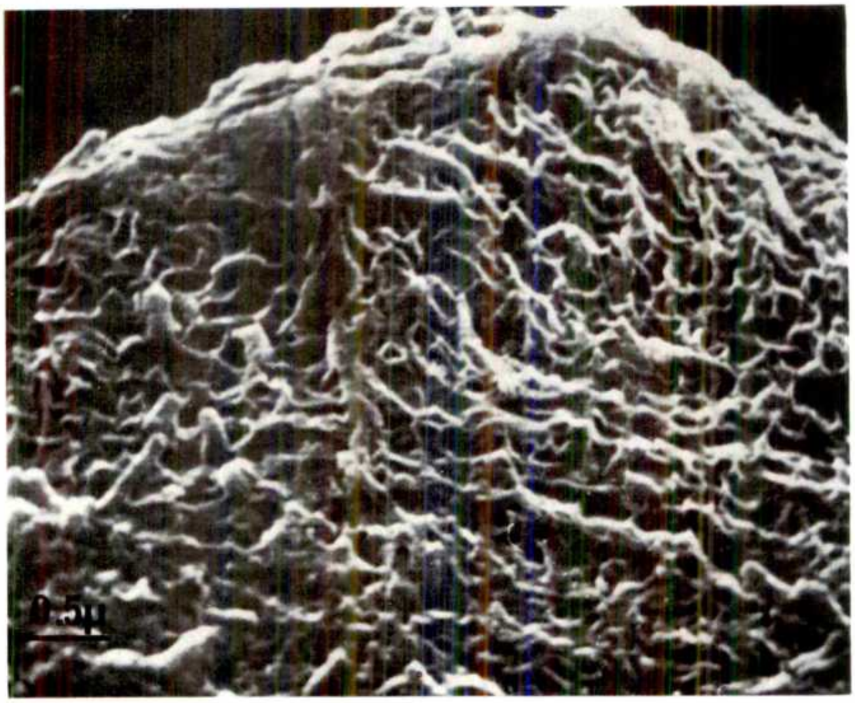

FIGURE 8. Scanning electron micrograph of a fixed, dehydrated, air dried corneocyte obtained after stripping the skin twenty times with adhesive tape. The surface is far more corrugated compared with Fig. 3.

cells, apparently several cells thick, which were seen to possess desmosomal remnants (Fig. 9). The constituent laminae were indistinct and somewhat irregular but were undoubtedly desmosomal in origin. Though this observation was rare in TEM, clumping of deeper corneocytes was more often observed by SEM. It is noteworthy that in Fig. 9 both the membranes and fibrillar portion appear to end abruptly. This was the only observation that could be interpreted as damage. All other observations on corneocytes from the deeper parts of the stratum corneum were as seen in the superficial cells.

## DISCUSSION

Conventional methods of tissue preparation may dislodge superficial horn and one advantage of the technique described is that it enables detailed inspection of large numbers of corneocytes from any depth within the stratum corneum (King *et al.*, 1979). One other significant advantage that the technique possesses is the ability to examine corneocytes from the same *in vivo* site by light microscopic and by scanning and transmission electron microscopic methods. Furthermore, the ability to first isolate the stratum corneum cells with minimal disturbance to their chemical composition and little (if any) physical damage permits the biochemical manipulation of corneocytes before examination. Thus, it is possible to determine the effects of pharmacological agents or physiological substances on the cellular components of the stratum corneum at any depth within this structure. A final advantage that may be mentioned concerns the TEM aspect of the study. Because the corneocytes are arranged in all planes in the pellet, it is possible to have a comprehensive view of both the corneocytes' membranes and cellular contents, since the cells are seen in both vertical and horizontal planes.

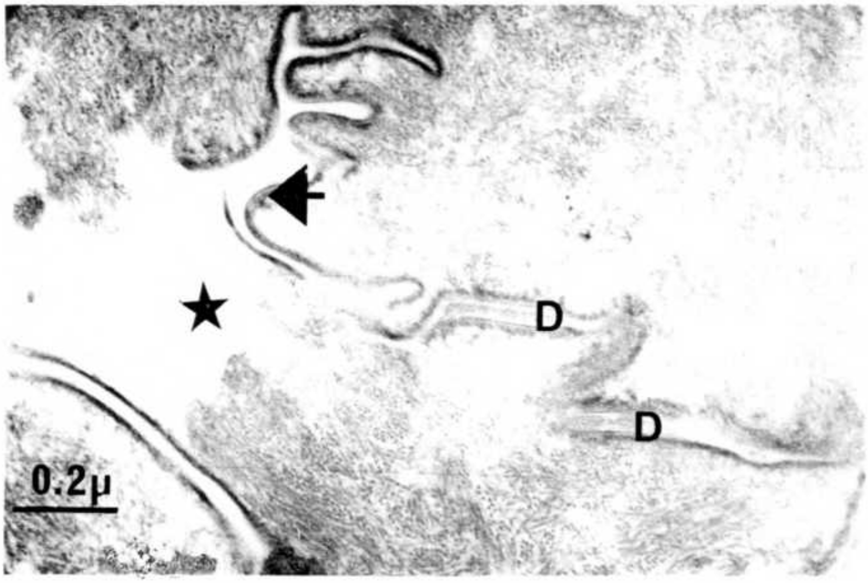

FIGURE 9. Section through corneocyte obtained after stripping the skin ten times with adhesive tape. Desmosomal remnants (D) are seen, though their constituent laminae are not distinct. Here and in other places the outer plasma membrane is distinct (arrows). The appearance at * could be interpreted as damage to the corneocytes.

There is one disadvantage which is difficult to evaluate. This concerns possible damage to the corneocyte membrane from the use of Triton X 100 detergent in the scrub fluid. The corneocytes cannot be easily removed without the inclusion of this detergent in the scrub fluid, and clearly it must in some way alter the physical properties of the horny cell. However, on direct examination of corneocytes removed by scraping from the surface of the skin without using a detergent, no obvious difference can be detected, and sections of superficial corneocytes from normally prepared biopsy material show little, if any, difference in ultrastructural appearance. Green (1977) reported that the plasma membrane proper of squames isolated from epidermal cell cultures was soluble in a mixture of 1% sodium dodecyl sulphate and 1% $\beta$ mercaptoethanol, but other factors affecting the visualization of the plasma membrane proper have to be considered (Raknerud, 1974).

The most complete study of the ultrastructure of the stratum corneum to date has been that of Brody (1969, 1970a, b). This author described the delicate internal fibrillar structure of the stratum corneum and described relatively electron dense corneocytes ('A' cells) with densely packed keratin fibrils and cells with electron lucent spaces ('B' cells), as well as intermediates between these types. The 'A' and 'B' cells were found to be irregularly distributed throughout the depth of the stratum corneum. Apart from the density of packing of the fibrils, Brody also found variability in the staining density of the keratin fibrils, with fibrils in the cells of the intermediate part of the stratum corneum staining more densely than those of the cells of either the basal or superficial strata. The structural and physiological significance of these slight morphological differences in corneocytes is at the moment unclear and our investigations have not revealed any fundamental differences between corneocytes.

Brody (1969) also commented on the modified plasma membrane of corneocytes. He reported that the plasma membrane proper was more opaque within, rather than between, the desmosomes and that the outer leaflet of the membrane proper was less opaque than the inner one. We agree with this view and the membrane proper is hardly discernible in most of our micrographs. However, the major component of the cornified membrane, Brody's broad zone, is very distinct and varies in appearance with varying planes of section. We do not agree with Brody that this zone exhibits stratification.

Raknerud (1974) studied plasma membrane modifications during keratinization of the hairless mouse using a variety of fixation and staining techniques. He found difficulty in distinguishing the outer lamina of the plasma membrane proper and stated that the inner lamina was always indistinguishable from the electron dense marginal layer (Brody's broad zone) in the upper part of the horny layer. In our micrographs of superficial corneocytes this also appears to be the case, but whether this is due to its inherent low contrast or its absence or its removal by solvents during the harvesting and processing of the cells is unknown.

In an SEM study of fixed, air dried biopsies, Orfanos (1971) reported that psoriatic and normal corneocytes could be differentiated by the presence of villi on the former. However, similar structures have also been reported in specimens of normal, unfixed and air dried corneocytes (Menten & Eisen, 1971), as well as in fixed, critical point dried material (Hashimoto & Kanzaki, 1975) and fixed, air dried corneocytes (King *et al.*, 1979). These specimens were tape stripped stratum corneum, whole skin biopsies and isolated cells respectively. In the present study fixed, critical point dried cells obtained after twenty sellotape strips have also demonstrated villous processes.

Our observation of pits on the surface of normal superficial corneocytes does not seem to have been previously reported. However, Orfanos (1971) observed 'pores' in association with the villi at deeper levels of psoriatic stratum corneum. These apparent discrepancies may be due in part to techniques of harvesting and processing stratum corneum. We do not know how many cell layers are removed with our technique, but all the cells must be very close to the surface. Tape stripping removes a variable amount of stratum corneum and, as Hashimoto & Kanzaki (1975) state, air drying may produce the villous nature seen by Menten & Eisen (1971). However, in Hashimoto & Kanzaki's (1975) critical point dried whole biopsies the most superficial corneocytes may have been lost in processing. The reticulum seen in unfixed non-dehydrated corneocytes also suggests that processing may remove or change the appearance of the components of the stratum corneum and this is also true in the case of biopsy material.

## ACKNOWLEDGMENTS

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# The experimental production of high-level intraepidermal splits

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## SUMMARY

The effects of trypsin at 37°C and 4°C on adhesion between keratinocytes in normal human epidermis were assessed by light and electron microscopy. The results provide evidence for the contribution of both extracellular and intracellular factors to keratinocyte adhesion and for changes in the nature of adhesion in the granular layer. Trypsinization at 4°C produced a high-level intraepidermal split identical in location and intercellular route to that observed in the staphylococcal scalded skin syndrome.

The current view of intercellular adhesion is that it represents the net outcome of a number of cell activities (reviewed in Marchase, Vosbeck & Roth, 1976; Edwards, 1977). Operationally, these can be divided into two groups, conveniently referred to by the terms 'stick' and 'grip' (Rees, Lloyd & Thom, 1977). Stick is defined as interactions between extracellular structures, such as exposed portions of cell membrane proteins, and is equivalent to the idea of a glue or intercellular cement. The term grip expresses the control of stick by intracellular cytoskeletal elements which influence overall cell rigidity, shape, and surface topography, and also exert transmembrane control on cell surface components such as those concerned with adhesion. Treatments which disrupt the cytoskeleton contribute to cell detachment by rendering cells more likely to deform and peel away from their contacts, and by increasing membrane fluidity, thus allowing adhesive configurations of cell surface proteins to disperse.

Experimental conditions have been devised which detach cells by influencing grip and stick to different extents (Rees *et al.*, 1977). One of these is the use of trypsin at different temperatures. At 37°C trypsin detaches cells by a combined action on grip and stick: cells round up, adhesive areas disperse and the cell peels away from the substrate. Trypsinization at 4°C, on the other hand, if sufficiently prolonged, detaches cells primarily by the dissolution of stick, and cells retain their flattened shape and rigidity. Comparison of the effects of trypsinization at 37°C and 4°C therefore provides a means of obtaining basic data on the contribution of grip and stick to overall cell adhesion.

These concepts are based on experiments to determine the factors contributing to the attachment of fibroblasts to an inert substrate (Rees *et al.*, 1977). Compared with this model system, epidermis is

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obviously complex. Adhesion between keratinocytes involves the presence of numerous desmosomes, as well as non-junctional adhesion. In addition, there is considerable evidence from morphological studies that the nature of keratinocyte adhesion changes in the course of epidermal differentiation (reviewed in Skerrow, 1978). However, the principle of considering adhesion as a net result of intracellular and extracellular interactions, of whatever kind, is a general one.

This paper reports the effects of trypsin at 37°C and 4°C on keratinocyte adhesion within normal human epidermis. The aim of the study was to assess the contribution of grip and stick to keratinocyte adhesion, and to determine whether the influence of these factors on adhesion alters in the course of epidermal differentiation.

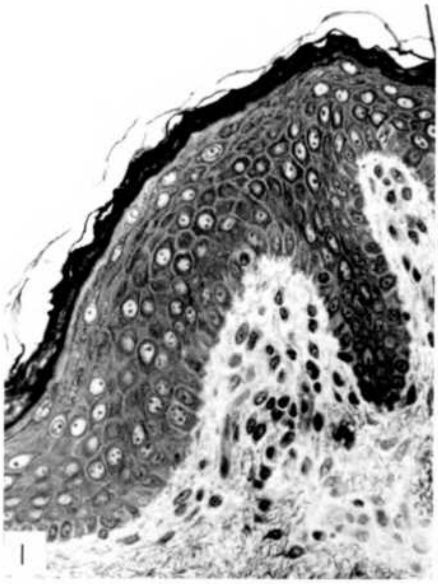
## MATERIALS AND METHODS

Normal human epidermis was obtained from circumcisions of children between 5 and 11 years of age. Tissue was collected in Eagle's minimal essential medium (MEM) containing antibiotic–antimycotic at 4°C and stored at 4°C for a maximum of 4 h before use. It was trimmed of connective tissue and cut into strips 2 mm in width. The strips were floated, dermis down, on a solution of 0·25% Difco trypsin in Eagle's MEM. In preliminary experiments 0·02% solutions of crystalline trypsin (Sigma grade III) in the same medium were also used. After incubation at 37°C for 2·5 h or at 4°C for 5 h, it was possible to peel the epidermis gently from the dermis. After incubation for times ranging from 30 min to 12 h, separated epidermis, or whole skin if short incubation times were involved, was placed in an 0·05% solution of trypsin-chymotrypsin inhibitor (Sigma grade II-O) at the same temperature as the trypsin solution and left for 30 min before fixation. Fixation was carried out for 1 h at room temperature, in 2·5% glutaraldehyde in phosphate buffer, pH 7·4. Tissue was postfixed in 1% osmium tetroxide in phosphate buffer for 1 h, dehydrated, and embedded in Epon 812. The epon blocks were sectioned for both light and electron microscopy. For light microscopy 1 μm sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead hydroxide and viewed in a Philips 301 electron microscope. Each experiment was performed four times, using tissue from a total of ten patients, and including control tissue subjected to identical treatment except for the omission of trypsin from the incubating solution.

In preliminary experiments, the ultimate effects of crystalline trypsin on keratinocyte adhesion were found to be the same as those produced by Difco trypsin, which is an impure enzyme preparation commonly used for dispersing epithelia into cells. However, crystalline trypsin required longer incubation times to produce the same effects on adhesion as Difco trypsin with approximately the same tryptic activity. In the course of this prolonged exposure, considerable damage to both intracellular membrane-bounded structures and to the plasma membrane were observed. In contrast, epidermis exposed to Difco trypsin showed little membrane damage. It seems likely that the effects of Difco trypsin are partially due to the presence of other enzymes. For the empirical reasons given above, Difco trypsin was used in all subsequent procedures.

## RESULTS

Control tissue was similar in appearance whether incubated at 4°C or 37°C over a period of 30 min to 12 h. Figure 1 is a light micrograph of skin incubated at 37°C for 6 h. It is well preserved, except that much of the stratum corneum has been lost. This is a common artefact resulting from the exposure of the tissue to lipid solvents during processing for electron microscopy. This section shows the changes in cell shape which characterize epidermal differentiation, from columnar basal cells to polyhedral spinous cells, then increased flattening throughout the upper spinous and granular layers, culminating in the extremely flattened cornified cell.



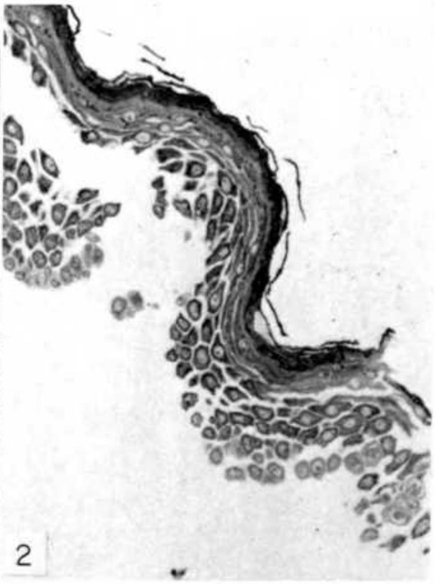


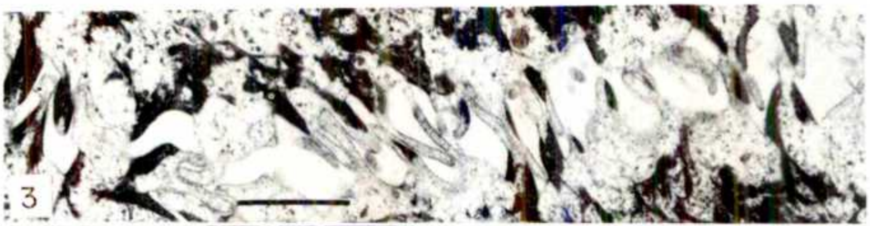
FIGURE 1. Control epidermis, incubated for 6 h at 37°C in Eagles MEM (× 360).

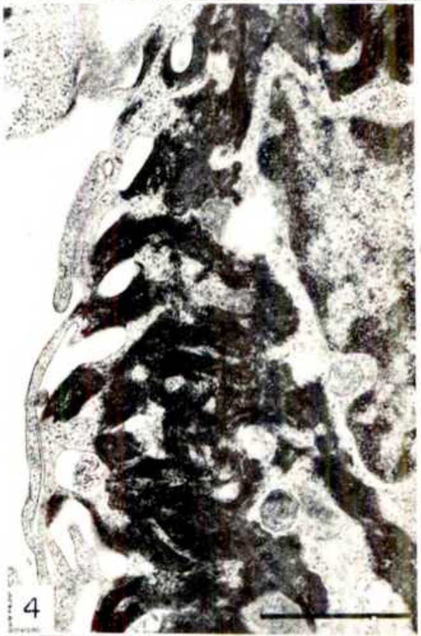
FIGURE 2. Trypsin at 37°C, 2·5 h. Note lack of detachment of granular cells, angular shapes of separated spinous cells (× 360).

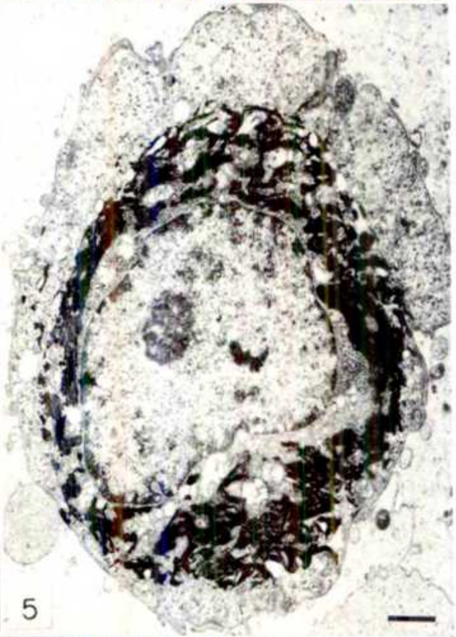
### *37°C trypsinization*

After exposure of epidermis to trypsin at 37°C for 2·5 h, considerable detachment of basal and spinous, but not granular, cells has occurred (Fig. 2). The only mechanical trauma experienced by tissue in these experiments, apart from that resulting from tissue handling, was the slight stretching due to epidermal–dermal peeling. For this reason, basal and spinous cells have largely retained their normal spatial relationships. At this stage (2·5 h) most detached basal cells are rounded, but spinous cells have retained their characteristic angular shapes. If epidermis is not peeled from the dermis, spontaneous detachment of basal and lower spinous cells is still observed after 2·5 h, but most upper spinous cells remain attached to the upper part of the epidermal tissue.

The changes leading to detachment are shown in Fig. 3, which shows part of the upper spinous layer in unpeeled whole skin exposed to trypsin for 2 h. The moderately electron dense material bisected by a midline, normally present in the desmosomal interspace, has been removed, leaving residual plaques with their attached tonofilaments. Most of these plaques are single, that is not paired with a corresponding plaque on the neighbouring cell. Few plaques have been internalized at this stage in trypsinization. In addition, there is no significant difference between the number of plaques present on the surfaces of the adjoining cells. This suggests that unpairing has resulted from the independent movement of residual plaques relative to the cell surface, presumably as a result of the increase in membrane fluidity which is known to be a consequence of trypsinization at 37°C. Further indications of this increased fluidity are the presence of numerous elongated protrusions (Figs 3 and 4) not present in control tissue at the same level. These protrusions enclose residual plaques and fuse at their free edges with the plasma membrane, forming vesicles (Fig. 4). The vesicles are displaced into the cell and the cell surface becomes rounded (Fig. 5). Detachment of basal and spinous cells occurs after the removal of interspace material, the increased protrusion of villi and the unpairing of plaques, but before the complete removal of plaques from the surface of the cell.







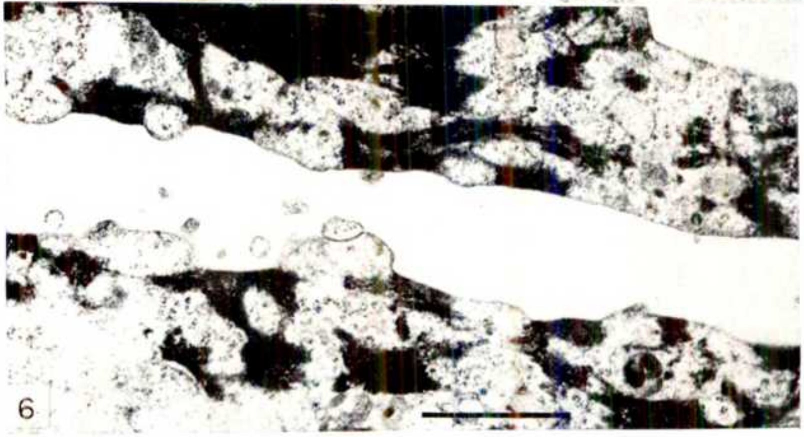


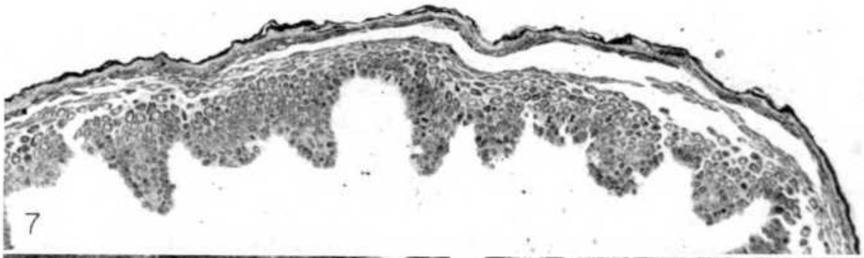
FIGURE 3. Trypsin at 37°C, 2 h. Spinous layer. Note complete removal of extracellular material at desmosomes, and predominance of single residual plaques. Bar 1 μm (× 14,800).

FIGURE 4. Trypsin at 37°C, 4 h. Spinous layer. Enclosure of residual plaques by cell protrusions which fuse with the plasma membrane to form vesicles. Bar 1 μm (× 19,800).

FIGURE 5. Trypsin at 37°C, 4 h. Spinous cell. Most residual plaques are internalized and the cell surface is rounded. Bar 1 μm (× 6,100).

FIGURE 6. Trypsin at 37°C, 12 h. Granular cells. Residual plaques are still on the cell surface, which has retained its smooth contour. Bar 1 μm (× 19,800).

Granular cells are still attached to each other after exposure to trypsin for 2·5 h and epidermal–dermal separation (Fig. 2). At this stage desmosomal interspace material is still present between granular cells, even at free edges of tissue fully exposed to trypsin. Detachment of granular cells requires longer trypsinization (4–5 h) with stirring. However, examination of fragments stirred for varying times does not reveal stages comparable to those observed in the basal and spinous layers. Removal of interspace material over parts of the cell surface is occasionally seen, with residual plaques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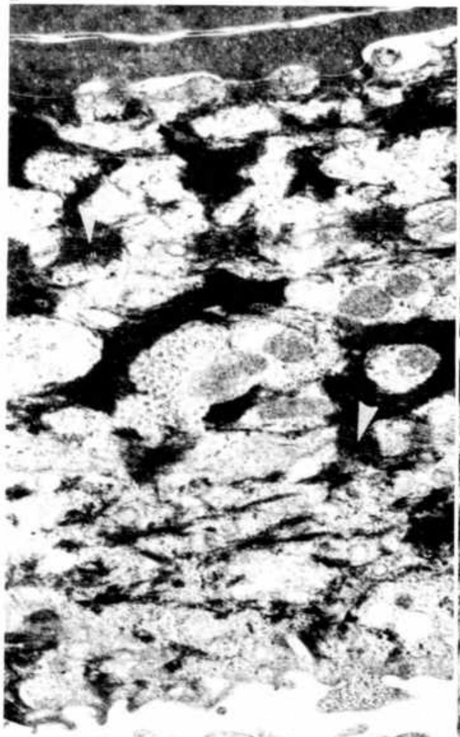
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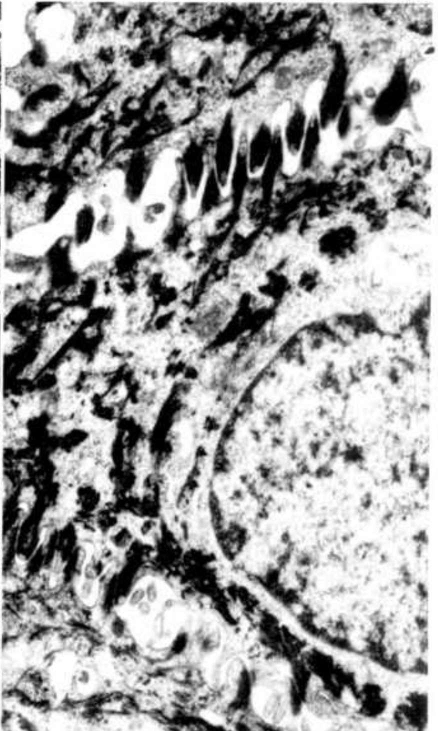
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FIGURE 7. Trypsin at 4 °C, 5·5 h. Note the continuous split at the spinous–granular layer interface (× 180).

FIGURE 8. Trypsin at 4 °C, 5·5 h. Above the split. Note the persistence of material in the desmosomal interspaces (arrows). Bar 1 μm (× 19,800).

FIGURE 9. Trypsin at 4 °C, 5·5 h. Below the split. Interspace material has been removed, but most residual plaques remain paired. Bar 1 μm (× 11,200).

remaining paired and non-junctional surfaces retaining the smooth contour seen *in situ*. Even in separated cells exposed to trypsin for 12 h, the cell periphery is smooth, with most residual plaques still on the surface, and the cell as a whole retaining its elongated shape (Fig. 6).

### 4°C *trypsinization*

Comparison of Figs 2 and 7 shows that the effects of trypsinization at 4°C on epidermis are strikingly different from those at 37°C. Progressive release of cells from the lower layers upwards has not occurred. Instead, focal high-level intraepidermal splits first appear in whole skin after trypsinization for 2 h. As trypsinization progresses, the split extends over larger areas, and in epidermis peeled from the dermis after 5·5 h, it is continuous throughout the tissue (Fig. 7). The separation is mainly located at the spinous–granular layer interface, occasionally extending into the spinous layer itself. A few upper spinous and lower granular cells become detached and lie free within the split, and these cells retain their flattened shape. However, above and below the split keratinocytes remain firmly attached to each other and are not separated by further trypsinization or by vigorous shaking. Even more drastic mechanical trauma, such as exposure to a Vibromix for 30 s, fails to detach cells and results merely in separation of the tissue along the split. The tissue above the split consists of stratum corneum and two to four layers of granular cells (Fig. 8). The desmosomes between these cells have not lost their interspace material, and this remains unchanged in appearance after trypsinization at 4°C for 12 h. In contrast, below the split in the basal and spinous layers, interspace material is removed, as it is at 37°C (Fig. 9). Intercellular spaces are widened, and focal areas of cell separation are seen. However, unlike the situation at 37°C (cf. Figs 3 and 9) most residual desmosomal plaques remain paired and on the surface of the cell, even after prolonged trypsinization. In addition, the normal topography of the cell surface, consisting at this level of short interdigitating projections, is maintained.

## DISCUSSION

The effects of trypsin at 38°C on chick embryo epithelia (Overton, 1973), at 4°C on rat buccal epithelium (Shimono & Clementi, 1977), and at 4°C on newborn rat epidermis (Fukuyama, Black & Epstein, 1974) have been reported previously. All these studies, which were at the ultrastructural level, agree that trypsin removes material from the desmosomal interspace and report varying degrees of plaque unpairing and internalization. However, in all cases additional mechanical action such as chopping, mincing and pipetting were used to further disperse trypsinized tissue, and for this reason no data on the differential action of trypsin at various levels within the epithelial strata were obtained. In addition, as comparisons between the effects of trypsinization at 4°C and 37°C were not their aim, control of temperature was not stringent. In the present study, which is the first report of the effects of trypsin on both the overall architecture of human epidermis and the ultrastructure of keratinocyte attachment, mechanical trauma was restricted to slight stretching, as this bears a closer relationship to the physiological forces acting on tissue during the early stages of bulla formation.

In the granular layer, the material in the desmosomal interspace is resistant to trypsin at 4°C and relatively resistant at 37°C (Figs 2 and 8). This result suggests that desmosomes within the granular layer, which are ultrastructurally modified, are also biochemically different, with respect to their interspace material, from desmosomes at earlier stages of epidermal differentiation. Detachment of these cells, even at 37°C, requires continuous mechanical action in addition to more prolonged trypsinization. It occurs without unpairing or internalization of plaques, protrusion of villous folds or loss of flattened cell shape (Fig. 6), suggesting that the mechanism is a type of brittle fracture through the intercellular space. These results indicate that both the granular cell as a whole, and its membrane, are more rigid than is the case with basal and spinous keratinocytes. The basis of this rigidity, unlike that in fibroblasts, is not sensitive to the action of trypsin at 37°C. This observation agrees with recent evidence for the rigidity, at 37°C, of the upper spinous and granular cell membranes of trypsinized keratinocytes from guinea-pig epidermis (Takigawa, Imamura & Ofuji, 1978). It is possible that the increase in granular cell membrane rigidity represents the early stages of formation of the rigid and chemically modified horny cell envelope (Matoltsy & Matoltsy, 1966). These changes would tend to increase the contribution of grip to keratinocyte adhesion in the granular layer and would effectively 'freeze' adhesive contacts. An increase in grip is also indicated by the ultrastructure of granular cell contacts in normal tissue; in contrast to the convoluted surfaces and variable intercellular spaces in the lower layers, granular cell non-junctional membranes are straight and closely apposed, with an average outer leaflet separation of only 15 nm (Skerrow, 1978). It seems probable that these modifications to both the desmosomal interspace and cell rigidity provide the basis for the observed resistance of granular cell adhesion to trypsin.

Detachment of basal and spinous cells with trypsin at 37°C involves not only the removal of cell surface material, including that in the desmosomal interspace, but also loss of normal cell surface topography and sliding apart of residual desmosomal plaques; in other words, a lesion of both stick and grip (Fig. 3). After trypsinization at 4°C, however, adhesion between cells at this level is maintained, although the treatment also removes interspace material (Fig. 9). Electron microscopy of this tissue indicates several mechanisms contributing to this continued attachment. Firstly, residual plaques which are known to be rigid (Overton, 1968) remain in register and provide considerable areas of grip. Secondly, the characteristic cell surface topography, consisting of numerous interdigitations, is preserved. As a result, cells are locked together in a three-dimensional network sufficiently strong to resist considerable mechanical trauma. These results are further evidence for the importance of grip to keratinocyte adhesion, both between non-junctional surfaces and at the desmosome.

The third factor which tends to oppose detachment of basal and spinous cells after trypsinization at 4°C is a direct consequence of their polyhedral shape, and the highly convoluted nature of their surfaces. As a result, desmosomes and non-junctional interdigitating surfaces are orientated randomly within the tissue. Shear in any direction may cause focal splits in the same plane, but these will not be propagated over a sufficient area of the cell surface to result in detachment. However, the contribution of this factor to keratinocyte adhesion does not remain constant throughout the basal and spinous layers. In the upper spinous layer cell peripheries begin to unfold, the numbers of interdigitations are reduced, and the cell becomes increasingly flattened in a plane parallel to the surface of the skin (Fig. 1). This process results in an increasing proportion of cell interfaces, including desmosomes, being orientated in the same plane: parallel with the surface of the skin and also parallel with the predominating direction of the shearing force when the skin is stretched. As flattening and surface unfolding progresses, the weakening of adhesion by removal of cell surface material with trypsin at 4°C, which gives rise to focal splits in the lower layers (Figs 7 and 9), would increasingly tend to produce splits propagated along this plane. This tendency would be maximal in the layer immediately below the typsin-resistant rigid granular layer described above.

This explanation for the location of the high-level intraepidermal split depends on considering a multiplicity of factors influencing keratinocyte adhesion, including desmosomal and non-junctional adhesion, extracellular molecules and cell processes controlling cell shape, rigidity and surface topography. The results reported in this paper provide further evidence for the changing contribution of these factors as keratinocytes differentiate. It is suggested that this pattern of adhesive change gives rise to a plane of weakness at the spinous-granular layer interface, at which the effect of agents which reduce stick but not grip will be preferentially expressed. On this view, it is not necessary to postulate the presence, at one level only, of specifically trypsin-sensitive adhesive molecules in the intercellular space. Indeed, if such molecules were present, a similar split might be expected to form at 37°C, but this is not observed.

The position of the split produced by trypsinization at 4°C and its intercellular route are identical to those observed in the staphylococcal scalded skin syndrome (recently reappraised by Lyell, 1979). In this bullous skin disease, a toxin produced by staphylococci and variously known as epidermolytic toxin and exfoliatin, produces splits at the spinous-granular layer interface. This effect can be demonstrated experimentally both *in vivo* (Melish & Glasgow, 1970) and *in vitro* (Elias *et al.*, 1974). A high level intraepidermal split has also been produced experimentally by treatment of mouse epidermis with ethylenediamine tetraacetic acid and subsequent traumatization by rubbing (Dimond, Erickson & Wuepper, 1976). The mechanism of cell detachment, however, differs from that of trypsin at 4°C, and exfoliatin, in that the cleavage plane passes through the peripheral cytoplasm, which is electron-lucent and oedematous. These observations could well be attributed to the disruptive effects of chelating agents on peripherally located intracellular cytoskeletal elements (Rees *et al.*, 1977). Epidermal microfilaments are particularly prominent, and organized into cortical bundles orientated parallel with the surface of the skin at a level corresponding with that of the high-level intracellular cleavage plane (Skerrow, 1978).

There is recent evidence that, unlike many exotoxins, exfoliatin does not bind to the cell surface (Baker, Dimond & Wuepper, 1978) and extensive searches have failed to reveal specific cell surface components, at this level, which would explain the site of action of the toxin (Elias *et al.*, 1975). The production of splits with the same location by trypsinization at 4°C casts further doubts on the necessity for postulating the presence, at a specific level only, of exfoliatin receptors or exfoliatin-sensitive adhesive material. It should be emphasized that it is not suggested that the action of exfoliatin is identical to that of trypsin at 4°C. Indeed, it has been shown that, *in vitro*, exfoliatin activity is not affected by trypsin inhibitors and effectively ceases at 4°C (Elias, Fritsch & Epstein, 1977). Rather, trypsin at 4°C and exfoliatin may both be examples of agents which weaken keratinocyte adhesion by a variety of actions on stick, whilst leaving the contribution of grip to adhesion unchanged. In this situation, for the reasons discussed above, the lesion is expressed as a high level intraepidermal split.

## ACKNOWLEDGMENT

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# The Ehlers-Danlos syndrome: an analysis of the structure of the collagen fibres of the skin

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## SUMMARY

The structure of the collagen fibres of the skin in the Ehlers-Danlos syndrome (ED-S) was studied in eight patients with ED-S Type I, three patients with ED-S Type II and three patients with the X-linked Type V.

The results show that the reducible cross-links are present and undergo the same maturation process to non-reducible cross-links as in normal skin. Transmission electron microscopy revealed a normal ultrastructure of the collagen fibrils. At a higher morphological level of organization scanning electron microscopy demonstrated progressive increase in fibre bundle disorder from the X-linked to mitis, to gravis, in which the fibres making up the large fibre bundles demonstrated a considerable inability to aggregate.

The Ehlers-Danlos syndrome (ED-S) is an uncommon, genetically determined disorder of connective tissue, classified on the basis of clinical, genetic and biochemical information into seven different sub-types.

The characteristic features of the syndrome are hypermobility of the joints, hyperextensibility and fragility of the skin (Fig. 1a–c) (McKusick, 1972; Beighton, 1970). The rarer forms are distinguished by the additional features listed in Table I. Types I, II and III are autosomal dominant disorders of unknown aetiology. Types IV, VI and VII are recessive and have well-defined collagen abnormalities (Pope *et al.*, 1975; Krane, Pinnell & Erbe, 1972; Pinnell *et al.*, 1972; Lichtenstein *et al.*, 1973). Type V is an X-linked syndrome in which there has been a recent report of a deficiency in the cross-linking enzyme, lysyl oxidase (Di Ferrante *et al.*, 1975).

The mechanical stability of the skin and the joints is directly related to the high tensile strength of the collagen fibres, and this property is dependent on the biosynthesis of a system of covalent cross-links between the tropocollagen molecules making up the fibres, and also on the size and integrity of the fibre bundles. It is therefore reasonable to assume that the abnormality of ED-S skin, and the instability of the joints, reflects either a basic defect in the collagen cross-linking or an abnormality of the fibre bundle.

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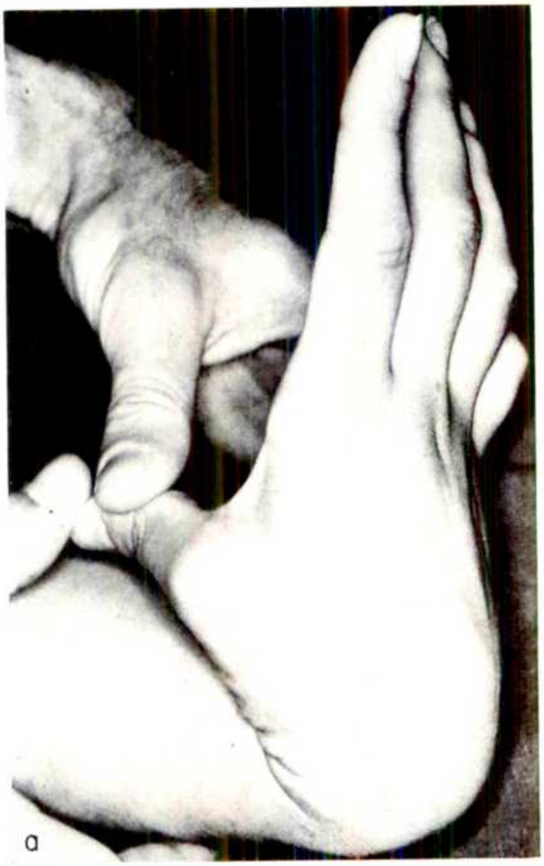

FIGURE 1a. Classical clinical feature of the Ehlers-Danlos syndrome: hypermobility of the joints.

The evidence for a primary abnormality in the collagen cross-links in ED-S is conflicting. There is no dramatic increase in the solubility of the skin (Harris & Sjoersdma, 1966) and the elastic modulus is no different from control subjects (Grahame & Beighton, 1969), both observations suggesting that the cross-linking of the collagen fibre is normal. However, the low breaking strength of the collagen fibre (Rollhauser, 1950) and the reported complete absence of the normal cross-links (Mechanic, 1972) both lend support to the hypothesis that the binding of the collagen fibre is defective.

We report here on the structure of collagen in fourteen patients (eight gravis, Type I; three mitis, Type II, and three X-linked, Type V). Particular emphasis has been given to studies of the cross-linking of collagen, and the scanning electron microscopy, in an attempt to define the role of cross-linking and the integrity of the fibre bundles.

## MATERIAL AND METHODS

Skin samples were obtained by consent from the forearm or thigh of fourteen patients, classified by one of us (PB). Normal skin controls matched for age, sex and site were obtained from autopsies performed on subjects who had no disease or medication known to interfere with the metabolism of connective tissue.

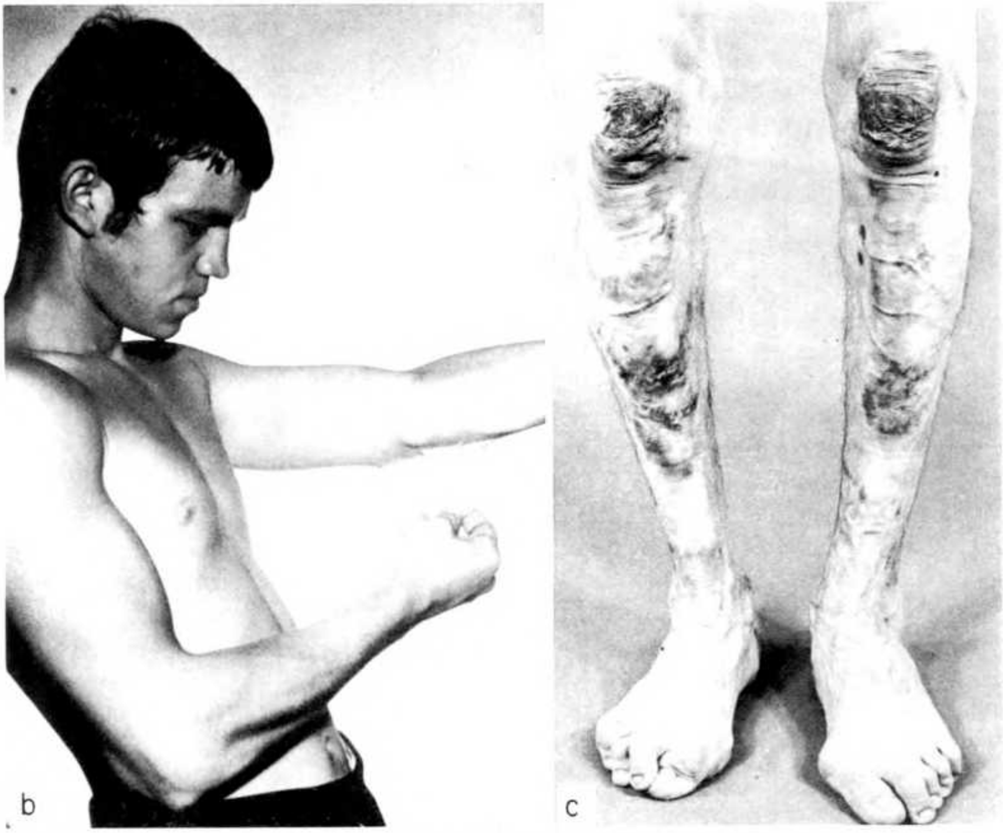

FIGURE 1. Classical clinical features of the Ehlers-Danlos syndrome: (b) hyperextensibility of the skin; (c) fragility of the skin with scarring. (Fig. 1b reproduced with permission of the *British Medical Journal*.)

TABLE 1. Types of the Ehlers-Danlos syndrome

| Nos | Type | Frequency (%) | Inheritance | Clinical features | Biochemistry |
|---|---|---|---|---|---|
| I | Gravis | 43 | Autosomal dominant | Classical severe | Unknown |
| II | Mitis | 35 | Autosomal dominant | Classical mild | Unknown |
| III | Benign hypermobile | 12 | Autosomal dominant | Joint hypermobility | Unknown |
| IV | Ecchymotic | 6 | Autosomal recessive | Lethal arterial rupture | Deficiency type III collagen |
| V | Sex-linked | 4 | Sex-linked | Mild classical | Lysyloxidase deficient (?) |
| VI | Ocular form | 2 | Autosomal recessive | Retinal detachment, Scoliosis | Lysyl hydroxylase deficient |
| VII | Arthrocalasis multiplex congenita | 3 | Autosomal recessive | Severe joint laxity, shortness | Procollagen peptidase deficiency |

*Transmission electron microscopy*

(a) *Embedded specimens.* The specimens were fixed in a 4% solution of glutaraldehyde for 4 h, and then washed in sodium cacodylate solution. The samples were then washed in a 1% buffered osmic acid, dehydrated through a series of absolute ethanol washes, cleared in propylene oxide, and embedded in epon. Sections were cut on a Porter Blum MT 1 ultra microtome, mounted on copper grids and examined in a Phillips EM 201.

(*b*) *Negative staining.* The specimens were briefly homogenized in physiological saline. A suspension of fibres was mixed with an equal volume of silico-tungstic acid (2% w/v, pH 8·4) and deposited on a grid covered with carbon coated collodion film. After allowing the suspension to settle, the excess liquid was removed and the grid allowed to dry (Tromans *et al.*, 1963).

(*c*) *Scanning electron microscopy.* The samples were freeze dried (Boyde, 1978) and mounted on the stud, coated with gold palladium, and then viewed with a Cambridge Stereoscan. Accelerating voltage was 15 kV throughout and the tilt angle of the specimen face to the beam was 44°.

(d) *Preparation of intact collagen fibres.* The skin biopsies were cleaned of adhering fat and muscular tissue, shredded with a scalpel, and washed with copious amounts of physiological saline, 0·9% NaCl pH 7·4, to remove soluble proteins and glycosaminoglycans.

(*e*) *Amino acid composition.* The intact collagen fibres were hydrolysed in glass-distilled constant boiling 6 N hydrochloric acid. The HCl was removed by evaporation *in vacuo* and a portion of the hydrolysate analysed on the Jeol automatic amino acid analyser JLC-6AH-DK.

(*f*) *Reduction of the intact collagen fibres.* The samples of intact collagen fibres were suspended in 0·9% NaCl pH 7·4 and reduced with tritiated potassium borohydride ($KB^3H_4$) diluted to 10 mCi per mmol with non-radioactive $KBH_4$. For direct comparison with age matched controls the $KB^3H_4$ was dissolved in saline (0°) and equal portions of the solution used to reduce the suspended fibres. The reaction was allowed to proceed for 1 h, after which time acetic acid was added to a final pH 4, the suspension dialysed against distilled water overnight, then freeze-dried and weighed.

(*g*) *Identification of reducible cross-links.* The weighed samples were hydrolysed in boiling 6 N HCl for 24 h, and the hydrolysate analysed by ion-exchange chromatography using the Technicon analyser with pyridine-formate buffers as described previously (Bailey, Peach & Fowler, 1970). The reducible components were identified in the collected effluent by determining the tritium activity of an aliquot from each fraction in the Packard Scintillation Counter using Brays solution.

Confirmation of the identity of the radioactive components was achieved by analysis against authentic samples on the long basic column (60 cm) of the Beckman 120 C amino acid analyser.

(*h*) *SDS Acrylamide gel electrophoresis.* Samples of the intact fibres were partially digested with pepsin (Worthington 10:1 collagen:pepsin) at 15°C for 24 h. The solubilized collagen was then denatured in 2% sodium dodecyl sulphate and the solution subjected to acrylamide gel electrophoresis using the technique previously described in detail (Sykes & Bailey, 1971) with the modification of interrupted electrophoresis (Sykes *et al.*, 1976) to separate the human $\alpha$I and $\alpha$III chains.

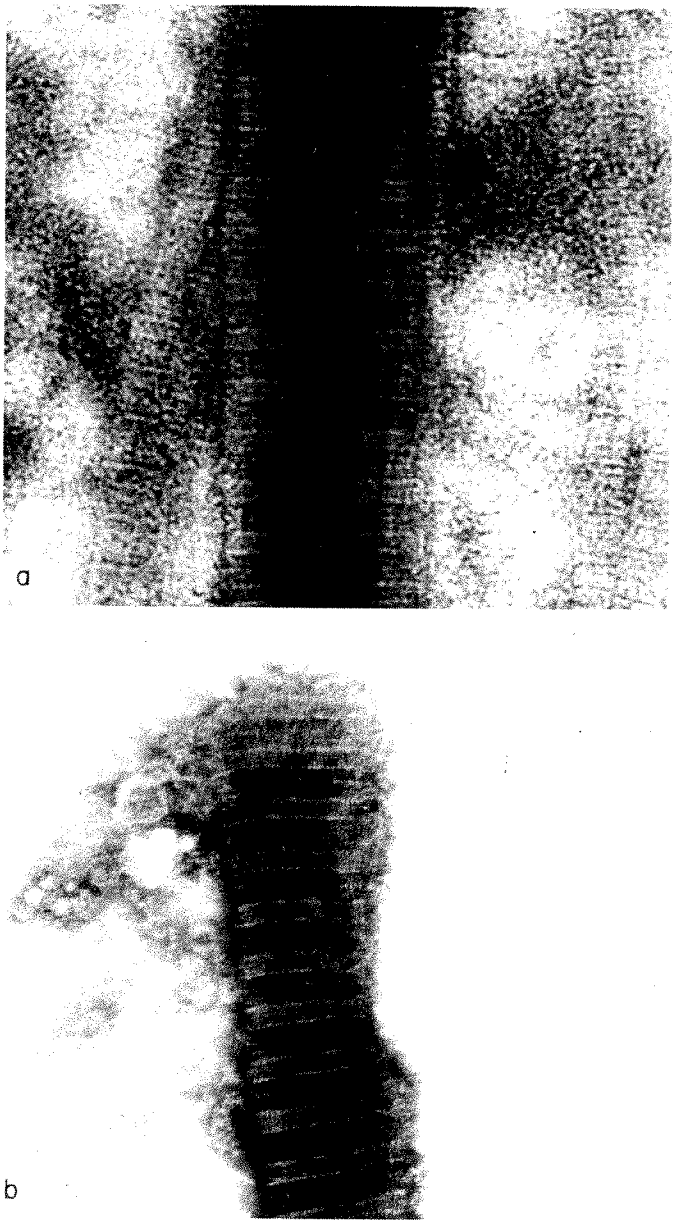

FIGURE 2. Transmission electron micrographs of collagen fibres. (a) normal skin fibre; (b) Ehlers-Danlos Type I (gravis). (Both ED-S Type II and V showed the normal axial periodicity and broken-ended fibres.)

## RESULTS

### *Transmission electron microscopy*

Examination of the embedded skin specimens revealed that the collagen fibres had a normal ultrastructure. In the negatively stained preparation the fibres possessed the usual axial periodicity, and the fibrils were uniformly organized, no difference from the control being detected. However, in those negatively stained preparations in which the fibres were briefly homogenized prior to examination, a significant number of broken-ended fibres were observed (Fig. 2b). No such sharp broken ends were ever observed with normal skin specimens (Fig. 2a). Examination of the cross-section of embedded fibres revealed little difference from normal, the fibres being perfectly round in cross-section and closely packed (Fig. 3). Determination of the mean fibre diameter revealed that the ED-S fibres were slightly thinner; gravis 81 ± 10 nm, mitis 78 ± 10 nm, x-linked 79 ± 10 nm compared to 101 ± 10 nm for normal fibres. In addition the fibre size distribution was wider in the ED-S than the normal. At lower magnifications examination of the packing of the fibres into fibre bundles again failed to reveal any significant differences, indicating that the disorganization occurs at a much higher level.

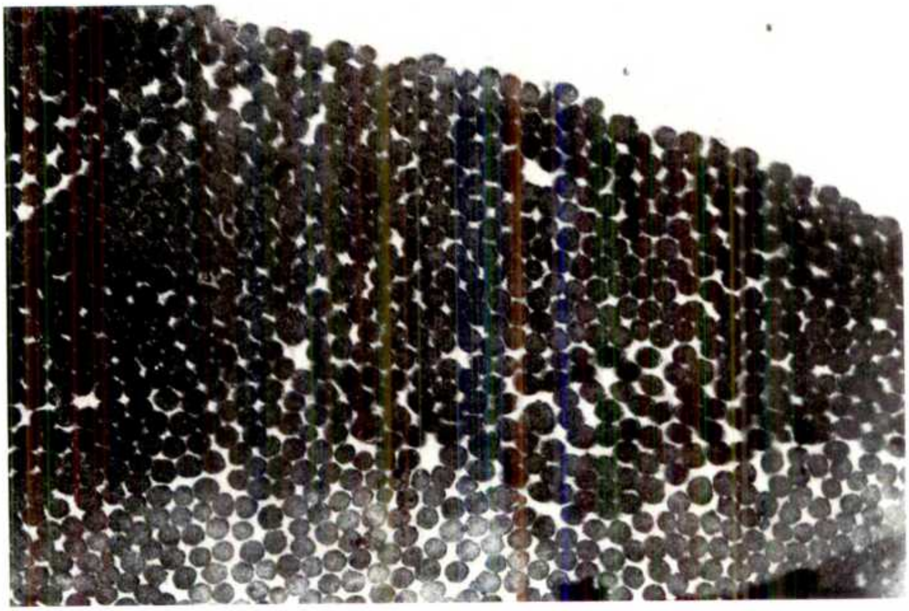

FIGURE 3. Cross-section of Ehlers-Danlos Type I (gravis) skin showing normal circular cross-sections of the individual fibres.

### *Scanning electron microscopy*

The fibre bundles from normal skin samples clearly had a 'preferred' direction although they were certainly less well orientated than in tissues such as Achilles tendon. The fibre bundles varied in size, were clearly separated from each other, and the fibres were closely packed within each bundle, resulting in a fairly smooth surface to the bundle (Fig. 4a).

In the mitis ED-S skin the bundles were smaller, and although still separate, were less closely packed such that the individual fibres within some of the bundles were now visible. The surface of most of these bundles was no longer smooth and the fibre bundles were not aligned in a preferred direction (Fig. 4b). The gravis (type I) showed grossly disorganized collagen fibres. The fibres appeared to have dried down to a flat 'feltwork' (Fig. 4c). This may be due to the very loose nature of the fibres within the bundles, such that on drying down they flatten out instead of keeping their shape and are therefore not easily discernible. There also appears to be a 'coating' of ground substance on the flattened out bundles. Examination of the X-linked ED-S skin samples showed a less extensive disorganization of the fibre bundles than the gravis, but more than the mitis. Further, the bundles appeared to be thinner than the normal and a high proportion of them were disorganized in that they were not tightly packed with smooth surfaces as in the case of the normal fibre bundles (Fig. 4d).

*Analysis of cross-links*

(*a*) *Skin of control subjects, and changes with age.* The chromatographic elution pattern of the radioactive components obtained from borotritide reduced human skin was found to be similar to that ob-

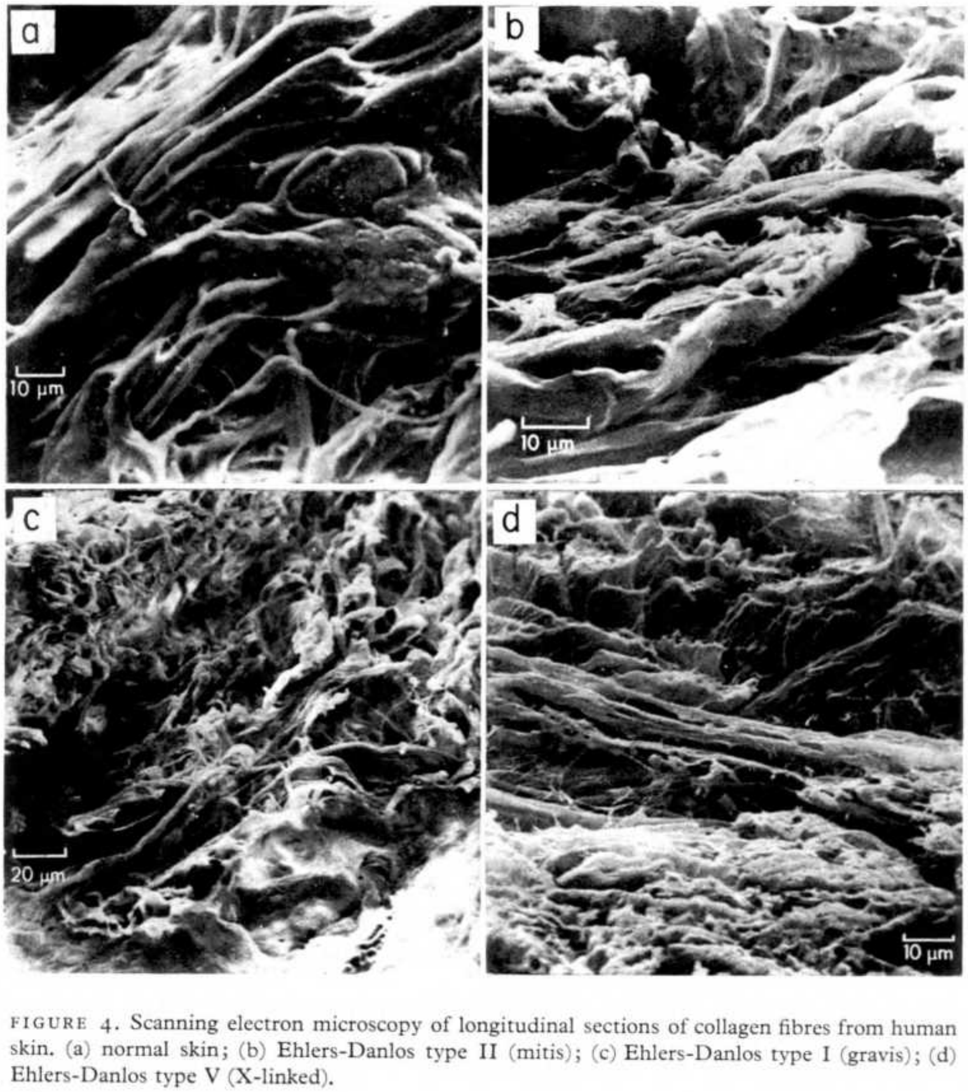

FIGURE 4. Scanning electron microscopy of longitudinal sections of collagen fibres from human skin. (a) normal skin; (b) Ehlers-Danlos type II (mitis); (c) Ehlers-Danlos type I (gravis); (d) Ehlers-Danlos type V (X-linked).

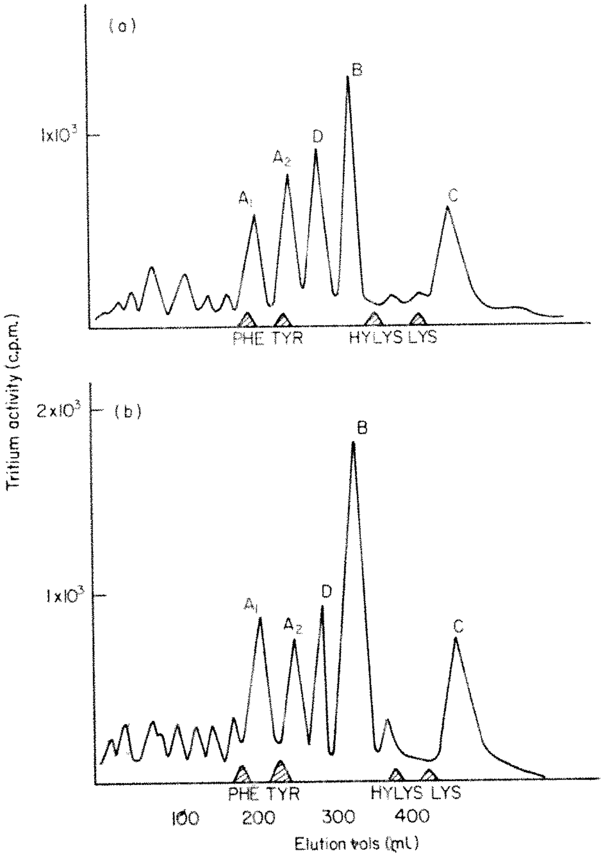

FIGURE 5. Typical distribution of radioactive components from acid hydrolysates of human skin reduced with tritiated potassium borohydride (a) normal skin collagen (11-year-old female); (b) ED-S type I skin collagen (11-year-old female). Peak B: Hydroxylysinonorleucine (OH-LNL). Peak C: Histidino-hydroxymerodesmosine (HHMD). Peak D: reduced Desmosine.

tained from calf skin, except that in the human skin a significant proportion of reduced desmosine derived from the reduction of the elastin cross-links was present (Fig. 5). Two major reduced cross-links were shown to be present in borohydride reduced human skin—hydroxylysinonorleucine (OH-LNL) and histidino-hydroxymerodesmosine (HHMD). On comparing specimens from subjects of increasing age, the proportion of these Schiff-base cross-links was found to decrease until, at about 20–25 years of age, the Schiff-bases had virtually disappeared. The proportion of HHMD, although decreasing with age, did so at a slower rate than OH-LNL, and the proportion of the former component was highly variable.

(*b*) *Analysis of reducible cross-links in Ehlers-Danlos skin collagen.* Specimens were examined from eight patients with the gravis form of ED-S, three with the mitis, three with the X-linked variety. The reduced cross-links hydroxylysinonorleucine (OH-LNL) and histidinohydroxymerodesmosine (HHMD) were found to be present in all samples (Fig. 5).

The proportion of these cross-links seemed to bear no relationship to the type or extent of the disease, but rather followed the age dependent curve of normal skin (Fig. 6).

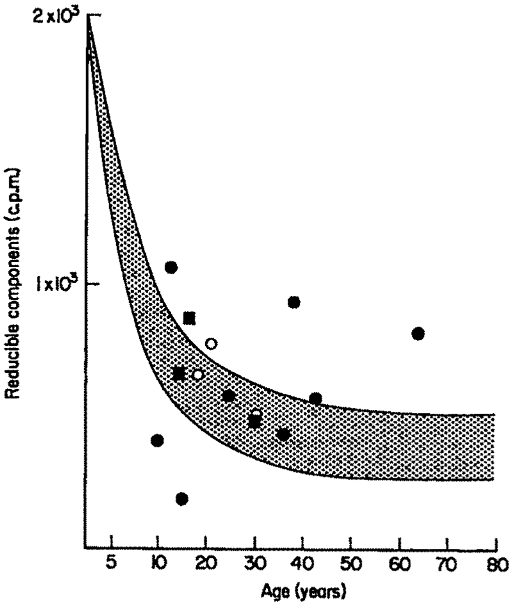

FIGURE 6. Summary of age-related changes of the amount of hydroxylysinonorleucine (OH-LNL) in skin collagen from Ehlers-Danlos patients. The shaded area represents the variation of OH-LNL in normal subjects. (●) Gravis; (○) mitis; (■) X-linked.

TABLE 2.

| | Gravis (Type I) (residues/1000) | Mitis (Type II) (residues/1000) | Sex-linked (Type V) (residues/1000) |
|---|---|---|---|
| Hydroxyproline | 86 | 84 | 94 |
| Aspartic Acid | 52 | 47 | 50 |
| Theonine | 20 | 18 | 18 |
| Serine | 39 | 34 | 35 |
| Glutamic | 77 | 71 | 76 |
| Proline | 121 | 123 | 125 |
| Glycine | 313 | 320 | 324 |
| Alanine | 102 | 116 | 104 |
| Valine | 25 | 38 | 24 |
| Methionine | 5 | 6 | 5 |
| Isoleucine | 13 | 14 | 12 |
| Leucine | 27 | 30 | 25 |
| Tyrosine | 5 | 6 | 4 |
| Phenylalanine | 14 | 15 | 12 |
| Hydroxylysine | 8 | 4 | 6 |
| Lysine | 36 | 28 | 31 |
| Histidine | 8 | 5 | 5 |
| Arginine | 49 | 45 | 51 |

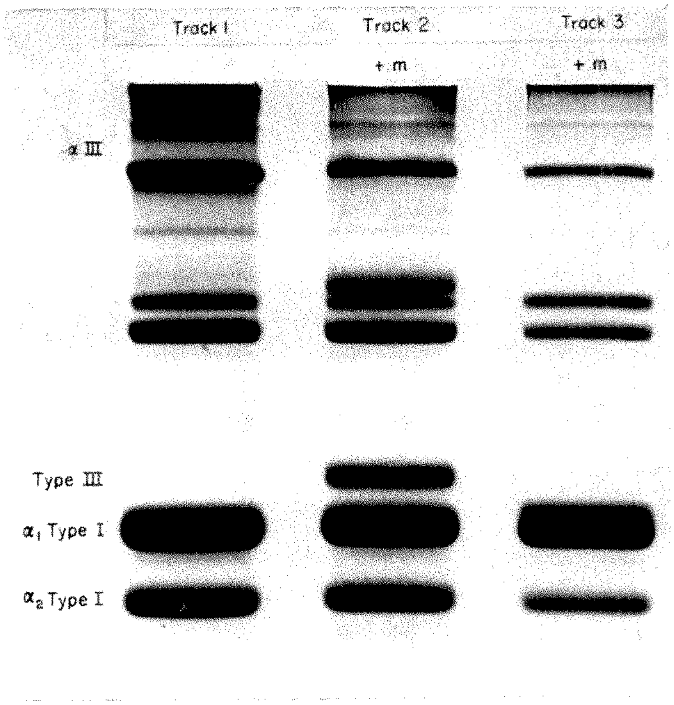

FIGURE 7. Typical SDS-polyacrylamide gel electrophoresis of human skin collagen from normal and Ehlers-Danlos subjects, showing the presence of the two major isomorphic forms of collagen Type I and Type III. Track 1 without β mercaptoethanol Type III runs as triple chain γ form. Track 2 interrupted technique, β mercaptoethanol added 10 min after start of electrophoresis. Track 3 pre-incubated with β mercaptoethanol, 1(I) and αIII bands run together. (Since ED-S and control were identical, a single pattern is shown.)

### *Amino acid composition*

In all the specimens examined from each of the ED-S types studied, amino acid compositions typical of collagen were obtained. No decrease in the proportion of hydroxylysine could be detected (Table 2).

### *SDS acrylamide gel electrophoresis*

Comparison of the gel electrophoretograms of the pepsin solubilized material from normal, gravis, mitis and X-linked samples failed to reveal any significant differences. All contained appreciable quantities of high molecular weight and components indicating extensive cross-linking of the α-chains. Similarly, Type III collagen was shown to be present in each sample (Fig. 7), which on a qualitative basis appeared to be present in the same concentration as the controls.

## DISCUSSION

The pattern of reducible cross-links in the dermal collagen of patients with ED-S types I, II and V was similar to that of normal skin. There were no new reducible components present, and the pattern showed the normal age related changes (Robins & Bailey, 1973) (Fig. 6). The only abnormality that could be detected was a slightly higher proportion of the reducible cross-links, but this could not be confirmed in absolute terms of cross-linking per molecule. The significance of this slightly higher proportion of reducible cross-links in mature subjects (Fig. 6) is debatable but suggests slower maturation rate. Accurate quantification of the cross-links is clearly required. The presence of the thermally stable cross-links observed in mature tissues was demonstrated by analysis of the electrophoretic pattern of SDS denatured material when higher molecular weight cross-linked components could be seen (Fig. 7). Further, we have recently shown normal levels of lysyloxidase activity in ED-S type V (Siegel *et al.*, 1979). These results, which demonstrate the presence of both reducible and stable cross-links, are at variance with the findings of Mechanic (1972) and Di Ferrante *et al.* (1975). We found no abnormality in hydroxylysine concentration or Type III collagen as observed in ED-S Type VI or Type IV (Pope *et al.*, 1975; Krane *et al.*, 1972; Pinnell *et al.*, 1972).

Transmission electron microscopy examination of the ED-S skin showed that although the fibres had a normal ultrastructure, there were a considerable number of broken-ended fibres (Fig. 2b). The proportion of the broken-ended fibres cannot be quantified but it is a clear demonstration of the fragility of the ED-S collagen fibres at the fibrillar level.

The diameters and the cross section of the collagen fibrils in the ED-S skin were perfectly normal. This contrasts with the disorganized cross-sections observed in the fragile skin fibrils from dermatosparactic cattle and sheep (Fjolstad & Helle, 1974; Bailey & Lapiere, 1973), a disease with the same biochemical defect as ED-S VII.

Scanning EM revealed significant differences from normal skin. The observed fragmentation of the fibres compared to controls probably accounts for the clinical manifestations of hyper-extensibility and hypermobility. Under tension, considerable extension will be required before the majority of the bundles are aligned in the direction of the tension, at which point they will be tightly packed and resist further extension. This would account for both the prolonged lag phase in the stress strain curve (Graham & Beighton, 1969) and the similarity of the elastic modulus to that of normal skin, once the point of resistance to the tension has been reached. If a defect in the cross-linking had been present, such as that in lathyrism or dermatosparaxis in cattle, continuous extension of the fibre would have occurred as the fibres slipped past one another, up to the point of rupture. In these situations no restoring force would be present and the effect would not be reversible whereas in ED-S subjects the skin returns to its normal position after hyperextension.

The ultimate breaking strength of the collagen fibre depends not only on the intermolecular cross-linking within the fibrils but also on the size and integrity of the fibre bundles. This is clearly shown in the increase in breaking strength, and fibre bundle size, with maintenance of a constant elastic modulus, during ageing of the mature animals (Gathercole & Keller, 1975). The lower breaking point of the ED-S fibres (Rollhauser, 1950) could therefore be due to the small size and irregular nature of the fibre bundle due to the lack of fibre–fibre interaction. The nature of this interaction is not at present known. It is possible that the proteoglycans play a role (Barker & Kennedy, 1969). Alternatively it may be a chemical defect of the collagen which results in an inability to react with the glycoprotein.

In summary, although these cross-linking and electron microscopic studies failed to demonstrate a primary chemical defect, they have provided support for the previously unsubstantiated proposal by Jansen (1955) that skin hyperextensibility in the ED-S is due to defective 'wicker work' of the collagen fibre bundles, the latter appearing to be normal in structure.

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## *Case Reports*

# Two cases of junctional epidermolysis bullosa (Herlitz-Pearson)

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## SUMMARY

Clinical details of one surviving and one fatal case of junctional epidermolysis bullosa, previously termed epidermolysis bullosa letalis-Herlitz, are reported and factors affecting prognosis are discussed.

## CASE REPORTS

### *Case* 1

G.L., male born in 1966 of unrelated parents. Blisters were noted over many areas of the body at birth. Recurrent episodes called 'impetigo' and loss of all finger and toe nails led to dermatological consultation and a diagnosis of epidermolysis bullosa (Dr R. Kelly, Melbourne Children's Hospital. Light microscopy showed sub-epidermal bullae). The patient is a well developed boy who has adapted well to his disabilities. General medical examination, psychological, psychiatric, endocrine and immunological assessments have not disclosed any abnormality. In particular, there is no clinical evidence of gut or renal involvement. Persistent involvement of the skin has been noted over the shins, calves and trunk, although initially the perioral areas and mucosae were most affected. Occasionally the groins were involved. Since the nails were shed, the finger tips have remained healed. The total area of skin involved at any time has never been greater than 10%. There has never been any ocular involvement or auditory symptoms. The primary dentition was recorded as normal apart from slight caries. The secondary dentition totally lacked enamel (Fig. 1a and b). Some of the clinical features are depicted in (Figs 2, 3 4a and b).

Early childhood was punctuated by episodes of infection and attacks of varicella and mumps which followed a normal course. Tonsillectomy was performed at the age of 6 without post-operative complications. Over the first 6 years, topical corticosteroids were applied as treatment to affected areas with or without topical antibiotics. The patient moved to Adelaide at the age of 7 and since then significant infections have been treated with systemic antibiotics according to predetermined *in vitro* sensitivity. Less significant infections have been treated with soaks of either potassium permanganate 1:10,000 or cetrimide 1:10,000. Recurrent attacks of balanitis led to stenosis of the prepuce requiring emergency surgical relief. Re-stenosis led to planned partial circumcision at the age of 10. Following this procedure, healing took place without scarring. There have been no further attacks of balanitis.

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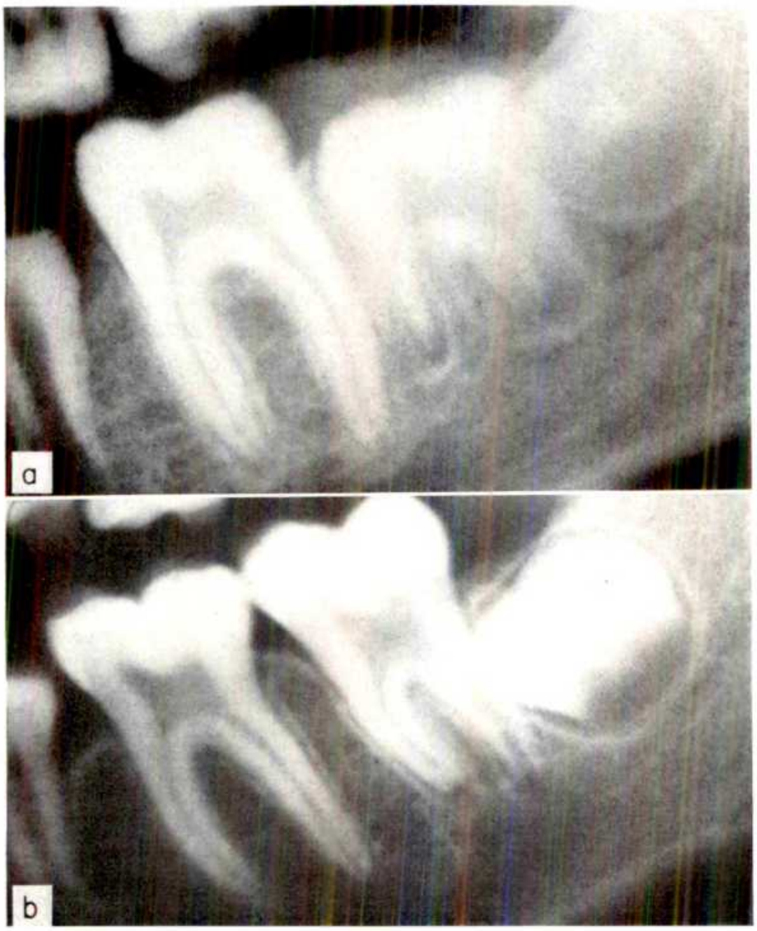

FIGURE 1. (a) Second dentition in JEB. Complete lack of enamel. (b) Non-affected sibling. The enamel cap is clearly visible.

Definitive diagnosis was established at the age of 10 years by electron microscopy on the grounds of defective hemidesmosomes and junctional separation (i.e. separation in the plane of the intermembrane space). This diagnosis was confirmed independently by Anton-Lamprecht in Heidelberg, West Germany. At the age of 10, collagenase levels were determined on uninvolved skin adjacent to the involved area on the shin. The collagenase activity was:

Fraction I: 111·75 ± 3·13 μg collagen hydrolysed/mg/min.

Fraction II: 49·46 ± 1·5 μg collagen hydrolysed/mg/min (Eisenberg, Steven & Schofield, 1978).

Full tissue typing and blood grouping were done on the patient and his parents (Fig. 5).

*Case 2*

M.S., female born 9 October 1977 of parents who were consanguineous (Fig. 6).

There is an unaffected male sibling. Delivery was normal and at birth extensive areas of denuded skin were noted over the trunk, buttocks, leg and perioral region including the palate. The skin around the nails also blistered. At day 7, 90% of the skin was involved (Fig. 7a and b). The child was

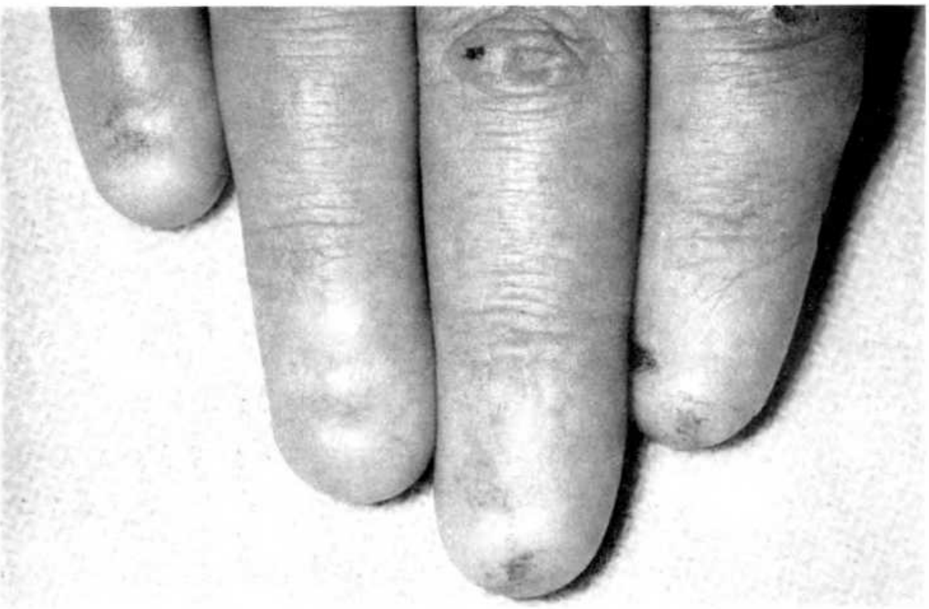

FIGURE 2. The nails are lost and there are several denuded and healing areas over the knuckles, but there is no webbing or scarring.

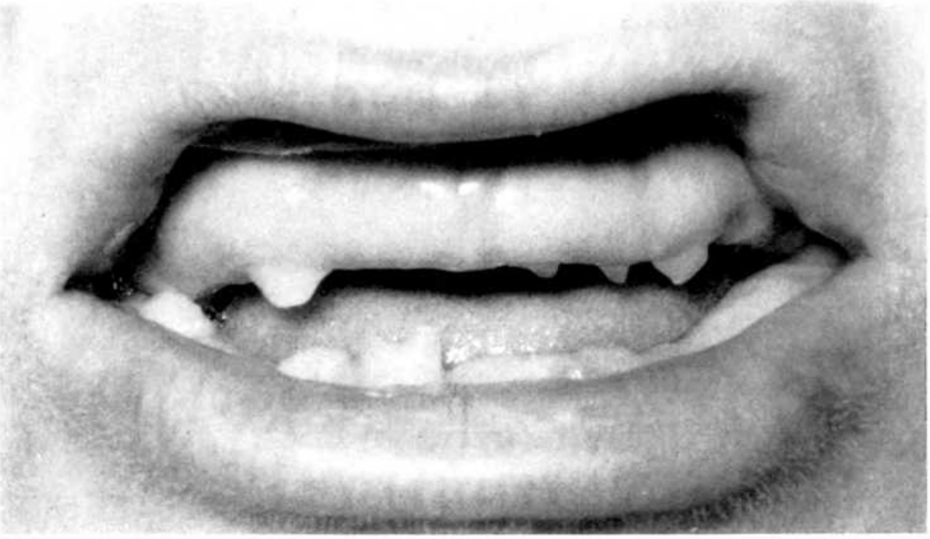

FIGURE 3. The second dentition is grossly defective.

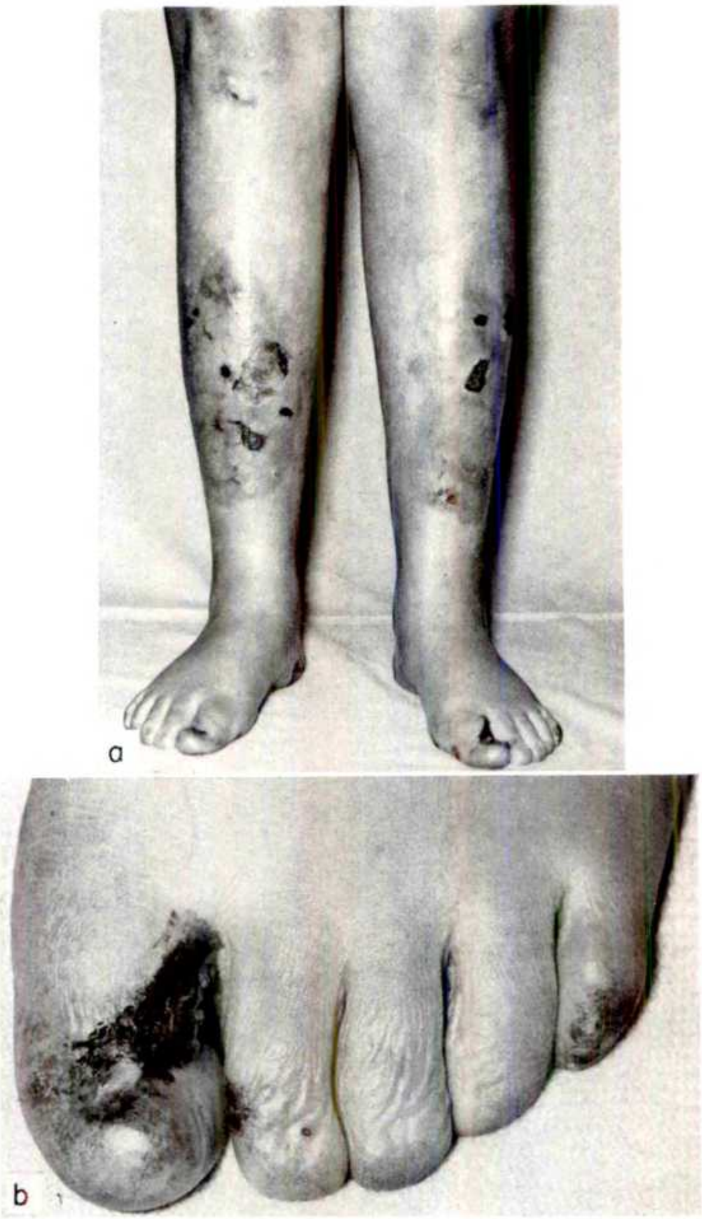

FIGURE 4. (a) The shins have been most traumatized, but still are not scarred. Note also the loss of toe nails seen more clearly in (b).

wrapped in gauze impregnated with white soft paraffin and reared in a temperature and humidity controlled crib. Haemoglobin was 16 g/dl and serum albumin 17 g/l. Initially the child improved, but although the skin healed, extensive blisters reformed in all areas. The nails were totally lost by 4 weeks. The haemoglobin steadily fell to 9 g/dl, serum albumin to 15 g/l and then 13 g/l.

| | Rh | | | | | | | KELL | | DUFFY | | KIDD | | MNS | | | | P | ABO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | D | E | c | e | $C^w$ | f | K | k | $Fy^a$ | $fy^b$ | $Jk^a$ | $Jk^b$ | M | N | S | s | $P_1$ | |
| G.L. | + | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | $A_1$ |
| Father | + | 0 | 0 | + | + | 0 | + | 0 | + | + | 0 | + | + | + | + | + | + | + | B |
| Mother | + | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | $A_1$ |

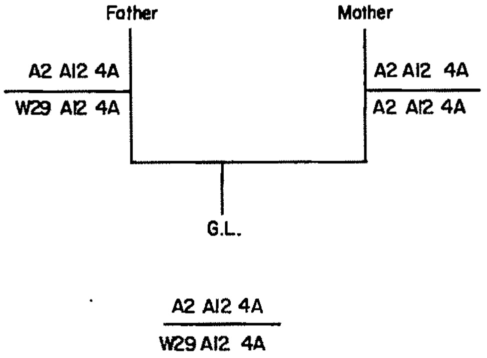

FIGURE 5. Tissue types and blood groups of Case 1 and his parents. The grandparents were not available for testing.

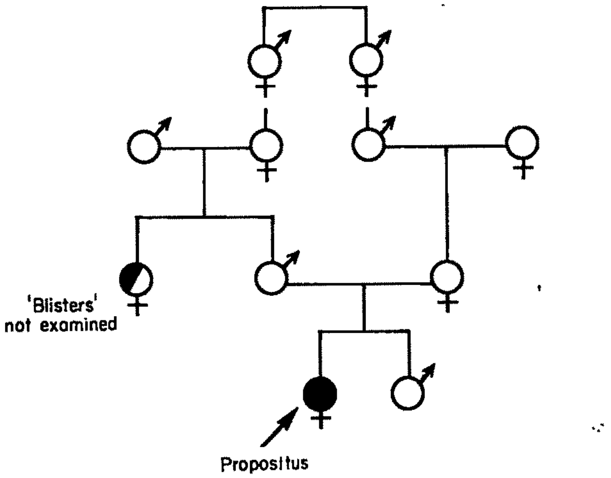

FIGURE 6. Family tree of Case 2.

Electron microscopy of a blister roof showed no associated dermis. There was oedema of the epidermis causing distension of the desmosomal ridges, but no evidence of splitting the cells and the desmosomes. There was no adherent granular basement membrane material on the blister roof. The base of the epidermis was smooth and traced the outline of the original dermal papillae, indicating very little disruption between adjacent basal cells.

Abdominal distension led to X-ray examination which disclosed fluid levels and dilated loops, suggesting peritonitis. Attacks of respiratory distress were a prominent clinical feature with X-ray findings of collapse and consolidation suggestive of aspiration pneumonia. The continuation of respiratory, bowel and skin problems led to death of the patient on 27 November 1977. Permission for autopsy was not granted.

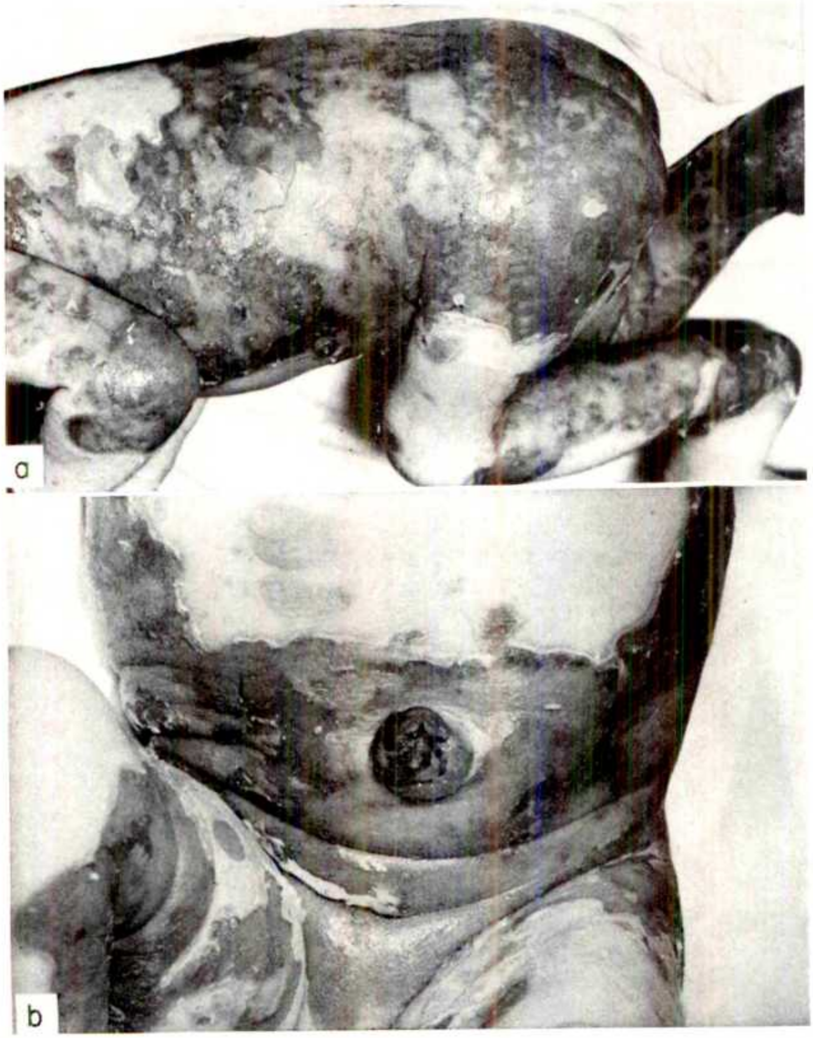

FIGURE 7. (a) Extensive skin loss. (b) Blister arising on uninflamed skin not separable from other severe EB at this age on appearance alone.

## DISCUSSION

To arrive at an accurate label for the disorder, a better understanding of the pathological defect is required. It has long been recognized that not all cases are fatal (Rosset, 1956) and junctional bullous epidermatosis of Pearson *et al.* (1974) may leave some doubt as to the identity of the condition with early reported cases under the title of epidermolysis bullosa hereditaria letalis-Herlitz. Junctional epidermolysis bullosa (Herlitz-Pearson) is therefore temporarily preferred.

From a review of the reported cases it can be said that the usual clinical picture is of a child born after an uneventful pregnancy, often to parents of known consanguinuity. At birth there are usually blisters or brown marks at the finger tips if nowhere else. Almost immediately, and certainly within the first few days, blisters extend to cover varying percentages of the body surface. All areas may be affected, but the sites of predilection, apart from the finger tips, are mouth (and mucosae), the shins, and the buttocks. The nails are lost early or deformed. There is an apparent difference between cases in which only the skin seems involved and those in which the bowel is also affected. The latter have difficulty in feeding and frequent bowel actions. They develop distension and visible peristalsis and quickly succumb. Slightly less severely affected cases show marked wasting. Acute sepsis is also a frequent occurrence and a notable cause of death. Almost all cases coming to autopsy show atelectasis, interstitial pneumonia, bronchopneumonia, aspiration pneumonia, pneumonitis, etc. This may reflect aspiration of regurgitated food secondary to the bowel defect, but the electron microscopic studies of Schachner, Lazarus & Dembitzer (1977) support the concept of respiratory, gastro-intestinal and genito-urinary involvement in severely affected cases. The outcome in those in whom only the skin is affected is much better, but even then problems of gross sepsis and persistent anaemia (Esterly & Hruby, 1974) and protein loss make the hold on life rather tenuous. If they survive these hazards long enough, the teeth will be seen to be affected, lacking enamel. However, they will not develop scarring or webbing of the digits, nor milia, as will cases of epidermolysis bullosa dystrophic recessive (Hallopeau-Siemens) (EBDR(H-S)).

It should be possible to establish the type of epidermolysis bullosa early in life in most cases on these clinical criteria alone. The family history is central and the possibility of sporadic mutants must at first be put aside. Within the dominant group, confusion is only likely between epidermolysis bullosa simplex (Köbner) (EBS(K)) and epidermolysis bullosa dystrophic dominant (Pasini) (EBDD(P)) at birth. The blistering is much more extensive in EBS (K) and shows no localization to prominences. EBDD(P), or Cockayne-Touraine (CT), may not develop symptoms until the second week of life or later. EBS(K) is widely affected at birth. Both types usually survive long enough for the presence or absence of scarring to decide the matter, even if the family history is hazy or unobtainable.

Light microscopy is not a satisfactory method of diagnosis. The position of separation in relation to the basement membrane is vital and methods of accurately staining the basement membrane zone are almost as technically demanding as preparation of a satisfactory block for electron microscopic studies. In any area of the world in which support systems are available to permit the child to survive the immediate situation, it is likely that electron microscope facilities will be reasonably close at hand. This technique allows accurate diagnosis in any circumstances such as mutation and hidden consanguinity following the studies of Pearson (1962) and the Heidelberg Group (Anton-Lamprecht & Schnyder, 1973; Hashimoto, Anton-Lamprecht & Meyburg, 1975; Hashimoto *et al.*, 1975, 1976a, b, c) (Table 1). As in Case 2, an initial attempt at separating EBDR and JEB in a gravely ill neonate can be made by electron microscopy of a blister roof from a newly formed blister. If this material does not contain granular basement membrane, extraordinary means are justified to maintain the existence of the child until formal biopsy can be tolerated and an exact diagnosis reached.

The data of Case 1 suggest that intellectual and somatic retardation and lethality are not necessarily part of JEB. His well-being discounts suggestions that JEB is severe EBDR. Immunological investigations do not suggest any associated defect, as does the uneventful recovery of the patient from varicella and mumps. The difficulty experienced by this patient with his many attacks of balanitis suggests that limited circumcision should probably follow the first attack of balanitis or may even be considered as an elective procedure early in life. The fragility of the skin should not weigh against the

TABLE 1. Diagnosis of epidermolysis bullosa in childhood

| | Recessive | | Dominant | | |
|---|---|---|---|---|---|
| | JEB | EBDR (HS) | EBDD(P & CT) | EBS(K) | *EBS(WC) |
| Onset of blisters | Birth | Birth | Delayed | Birth | Delayed |
| Predilection sites | Perioral, shins, trunk, finger-tips | Extremities, pressure points | Extremities | Whole body | Hands and Feet |
| Milia | Absent | Frequent | In about 50% | Absent | Absent |
| Nails | Shed early | Dystrophic | Dystrophic | Unaffected | Unaffected |
| Teeth | (Case 1)<br>Primary unaffected, secondary lack enamel | Caries, hypoplastic enamel, missing teeth, misplaced teeth | Frequently affected | Usually unaffected | Unaffected |
| Electron microscopic findings for skin | Sparse rudimentary hemidesmosomes, separation in the intermembrane space | Dermal separation, anchoring fibril/collagen fibre defect | Dermal separation, anchoring fibrils missing or rudimentary | Separation above basement membrane | Separation above basement membrane, basal cell vacuolation |

*EBS(WC) = epidermolysis bullosa simplex (Weber-Cockayne).

performance of surgical procedures in these patients, since their skin heals well without constricting or hypertrophic scarring.

In Case 1 the dental changes differ slightly from those previously described (Arwill, Bergenholtz & Olsson, 1965). In Arwill *et al.'s* two cases the enamel was present, but enevenly distributed, being thicker on the lingual than the buccal aspect. The formation of enamel is dependent upon the presence of the stratum intermedium of the enamel organ. Only that area of the ectodermally derived internal enamel epithelium (ameloblasts), covered by this condensed layer of squamous cells, is organized into the formation of prismatic enamel matrix. In amelogenesis imperfecta (occasionally reported in relatives of patients with JEB) and in JEB the enamel is either not formed (Case 1) or is globular (Gardner & Hudson, 1975). The changes in the teeth after birth have been reported by Brain & Wigglesworth (1968). They suggest that changes in dentine may relate to malnutrition in those patients who survive more than 10 days. These changes are quite different from the commonest dental lesions in dystrophic epidermolysis bullosa (Fig. 8) namely, simple caries, possibly partly due to inadequate cleansing through fear of blister formation. Other dental anomalies collected by Arwill *et al.* (1965) from the literature include rudimentary teeth, diminished numbers of teeth, including total absence and unevenly spaced teeth. Unfortunately it is not always clear in the original reports that the cases have been accurately classified. As the secondary dentition emerges, individual acrylic caps could be fashioned to preserve the enamel-free teeth as an alternative to restoration, or steel caps as suggested by Crawford, Burkes & Briggaman (1976). Our patient has deteriorated too far for this procedure to be a practical consideration. As a result, the patient has difficulty in eating many hard foods. Standard dentures would produce too much disability through friction and blister formation.

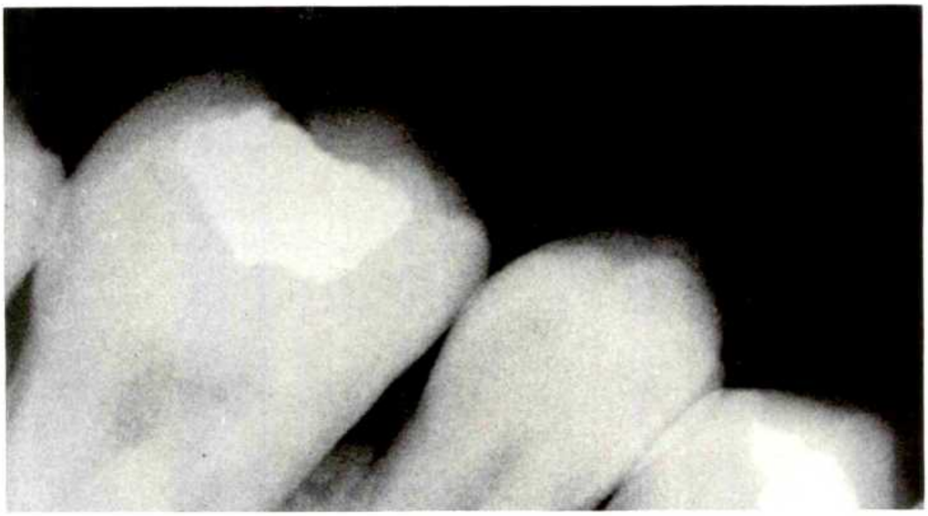

FIGURE 8. Second dentition in EBDR(HS). Normal enamel cap. Tooth affected by caries and filled.

There were no ocular changes in either Case 1 or 2; indeed Aurora, Madhavan & Rao (1975) reported the only case with eye changes. The unavailability of electron microscopy and absence of other reported cases indicate caution in accepting this case as accurately classified.

The raised collagenase levels are of interest, but cannot at present be used to separate the various types of epidermolysis bullosa (Bauer, Gedde-Dahl & Eisen, 1977). It is premature to speculate on the meaning of these levels until further studies have been done in similar patients. Dilantin, a known collagenase suppressor *in vitro*, was given speculatively to Case 1 on the suggestion of Eisenberg. The blood level of dilantin was monitored and kept at the ordinary therapeutic level for epileptics (40–80 $\mu$mol/l). Over an 18-month course, no difference was noted in the manifestations of the disorder. Similarly, vitamin E in a dose of 500 mg a day produced no clinical improvement, even though continued for approximately 1 year. The tissue typing data are presented without comment. Much more similar data will be necessary before a linkage (if any) becomes apparent. The patient has noticed that early opening of newly formed blisters speeds resolution. The only other factor associated with significant improvement remains the avoidance of trauma and adequate control of skin infections. With current bacteriological techniques and antibiotics, sepsis should not be the problem it once was.

The problems encountered in Case 2 highlight the need for an 'intensive care' approach. A minimum of handling will limit skin loss and the technique of amniotic explants used to cover the skin temporarily in patients with burns could be investigated.

The most obvious difference between these two cases is that the survivor has never had more than 10% of his skin affected, nor evidence of other system involvement, whereas the non-survivor had 90% of the skin involved at one time at least, with unmeasured bowel and possible lung involvement.

The major determinant of survival in JEB is the ability of the child to conserve protein, fluid and minerals. With adequate homeostasis, control of infection is a difficulty which is slightly more readily overcome.

In future cases the percentage of skin involvement and the net protein, mineral and fluid losses should be measured and charted and correlated with survival time. Attempts should be made to separate losses from skin, gut and respiratory systems. Reporting of these parameters should allow the formulation of prognostic guide lines. A vigorous approach to the maintenance of life may even improve prognosis to a point where 'letalis' may be omitted from the name of the disorder.

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*British Journal of Dermatology* (1980) **102,** 109.

# Myelofibrosis with skin lesions

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## SUMMARY

An elderly male patient with primary myelofibrosis developed many papules on the trunk and later numerous subcutaneous nodules on the face. Histologically these lesions showed the features of extramedullary haematopoiesis consisting of all the three components, i.e. myeloid, erythroid and megakaryocytic elements, findings never reported in the skin.

Haematopoiesis takes place in the skin only in early embryonal life (Popoff & Popoff, 1958). Thus, infiltration of the skin by immature myeloid and erythroid cells usually does not occur, except for a rare case of erythroleukaemia (Belisario, McGovern & Dawson, 1958).

Myelofibrosis is regarded as belonging to the myeloproliferative disorders like leukaemia and polycythaemia vera. It differs from the latter by showing proliferation of the connective tissue cells of the bone marrow accompanied by active proliferation of potential bone marrow elements in other organs, particularly in the spleen, liver and lymph nodes. Therefore, clinically the syndrome of myelometaplasia with myelofibrosis is characterized by (1) splenomegaly often with hepatomegaly; (2) replacement of the bone marrow by fibrous tissue; (3) leukoerythroblastic peripheral blood picture; and (4) extramedullary haematopoiesis. Although this haematopoiesis occurs in various organs, the skin is rarely involved (Pitcock, *et al.*, 1962). There have been only a few reports on such cases in the past (Kuo, Uhlemann & Reinhard, 1976; Ortonne, Jeune & Perrot, 1977).

The purpose of this paper is to report a case with primary myelofibrosis associated with numerous skin lesions that histologically exhibited the typical features of extramedullary haematopoiesis.

## CASE REPORT

A 74-year-old Japanese man developed general weakness and abdominal distension in January 1975 and pallor of the face a few months later. From May to July 1975, he was admitted to the Kyoto University Hospital, being diagnosed as having myelofibrosis. In December of the same year he was readmitted because of gradual deterioration of the condition and development of an asymptomatic papular eruption on the trunk.

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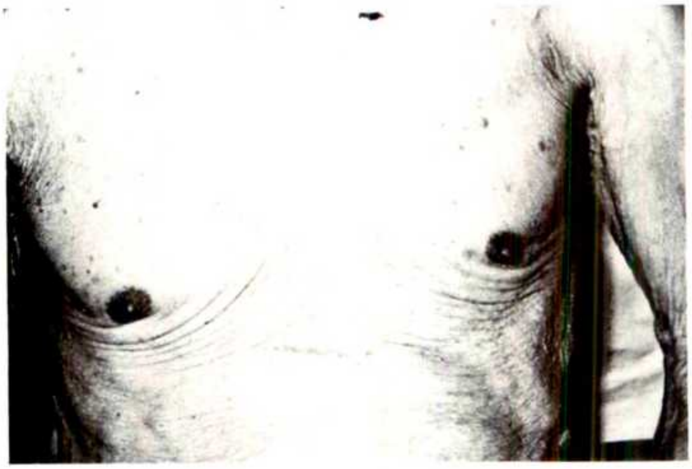

FIGURE 1. Papular lesions on the chest and abdomen.

Physical examination at that time revealed numerous red solid papules 1–5 mm in diameter scattered over the chest and abdomen (Fig. 1) as well as enlarged liver and spleen, palpable four and five fingerbreadths below the right and left costal margins, respectively.

Haematological studies revealed the following values: haemoglobin 9·2 g/dl; RBC $3{\cdot}2 \times 10^6/\text{mm}^3$; 65 erythroblasts/100 WBC; WBC $27{,}400/\text{mm}^3$; differential cell count, fifty-four premyelocytes,

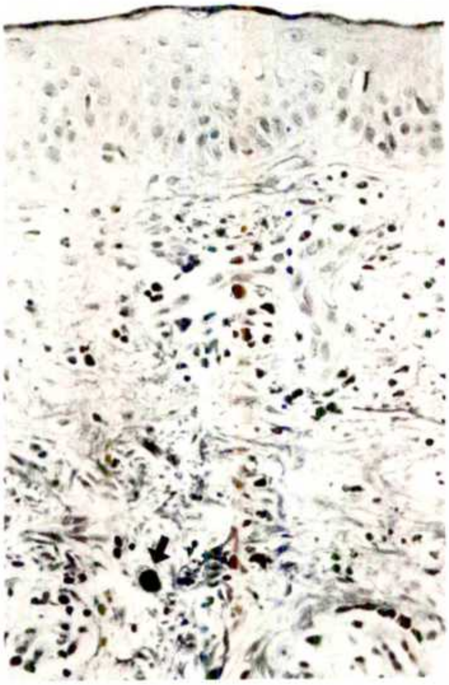

FIGURE 2. Skin biopsy specimen showing extramedullary haematopoiesis in the dermis in which a megakaryocyte is observable (arrow) in addition to myeloid and erythroid cells (H & E, × 80).

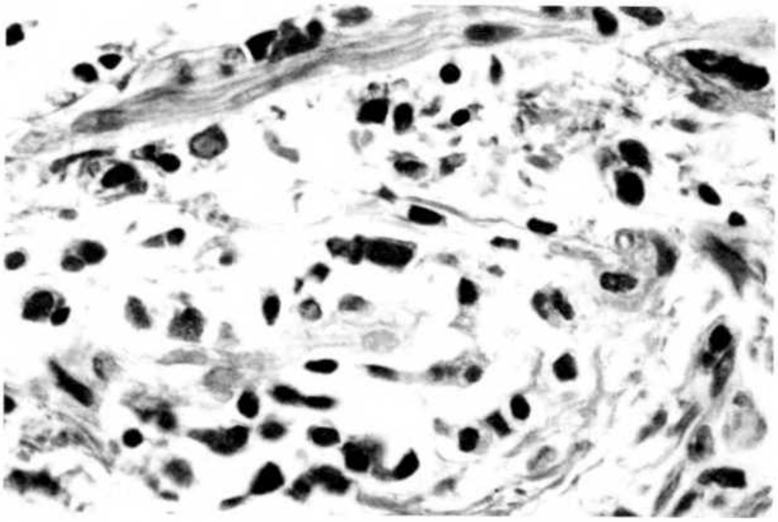

FIGURE 3. Higher magnification of the perivascular region showing nucleated red blood cells with dark, round pyknotic nuclei and large myeloid cells (H & E, × 360).

one myelocyte, one metamyelocyte, eleven bands, seven neutrophils, twenty-four lymphocytes and two monocytes.

Biopsy was performed on two of these papular lesions on different occasions; both specimens showed similar findings. In the dermis there was a polymorphic cellular infiltration consisting of myeloid, erythroid and even megakaryocytic cellular elements (Fig. 2). The cellular infiltrate was particularly rich in the perivascular area. Higher magnification clearly revealed clusters of erythroid cells in various stages of maturation with dark, round, pyknotic nuclei and a rim of eosinophilic cytoplasm intermingled with myeloid cells with much larger nuclei and ample, occasionally granulated cytoplasm (Fig. 3).

During the next few months the papules increased in number and involved the lower extremities. Moreover, numerous subcutaneous nodules began to appear on the face, attaining the size of a hen's egg. The patient died in March 1976 following progressive exacerbation of the general condition.

At autopsy, there was fibrosis of the bone marrow, splenomegaly, hepatomegaly, generalized lymphadenopathy, multiple nodules in the stomach and numerous cutaneous papular and nodular lesions. They all displayed the histological features of extramedullary haematopoiesis consisting of myeloid, erythroid and megakaryocytic series. Furthermore, a leukaemic cell infiltration was demonstrated in the muscle, lung and epididymis.

## DISCUSSION

This patient showed numerous skin lesions accompanied by the histological features of extramedullary haematopoiesis as well as many classical characteristics of primary myelofibrosis.

A review of the literature revealed that extramedullary haematopoiesis in the skin of patients with myelofibrosis has been described in four cases (Table 1). Only the case reported by Kuo *et al.* (1976) showed histologically both erythroid and myeloid cells. In other cases the infiltrate consisted entirely of myeloid cells and not a mixed infiltrate as is usually seen in other organs such as the liver and spleen (Pitcock *et al.*, 1962).

TABLE 1. Extramedullary haematopoiesis in the skin

| Case no. | Authors | Age (years) | Sex | Skin lesions | Consisting cellular element |
|---|---|---|---|---|---|
| 1 | Pitcock *et al.* (1962) | ? | ? | ? | Myeloid series |
| 2 | Kuo *et al.* (1976) | 66 | M | Nodule | Myeloid and erythroid series |
| 3 | Ortonne *et al.* (1977) | 62 | M | Erythematous infiltration | Myeloid series |
| 4 | | 68 | F | Red papules | Myeloid series |
| 5 (present case) | Tagami *et al.* (1979) | 74 | M | Red papules and nodules | Myeloid, erythroid and megakaryocytic series |

The present case is unique not only in that there were a variety of cutaneous lesions but in that they demonstrated all the three components of haematopoiesis, i.e. myeloid, erythroid and even megakaryocytic elements. Although coexistence of myeloid and erythroid cells was also found in a rare case of erythroleukaemia (Belisario *et al.*, 1958), to our knowledge this is the first case in which megakaryocytes have been demonstrated in the cellular infiltrate of the skin.

## ACKNOWLEDGMENT

The authors are grateful to Dr H.Shirasawa (Professor of the Department of Pathology, Hamamatsu University School of Medicine) for his help in histological assessment.

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# Contact allergy to 8-methoxypsoralen

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## SUMMARY

A 20-year-old male patient was treated topically with 8-methoxypsoralen and long-wave ultraviolet light (UV-A) for alopecia areata. He developed contact allergy to 8-methoxypsoralen after the sixth treatment (day 17), which was verified by patch testing and histopathology.

One of the most widely used phototoxic compounds for the treatment of a variety of dermatoses is 8-methoxypsoralen (8-MOP). 8-MOP is particularly useful for the treatment of psoriasis and cutaneous lymphomas. It can be applied topically or systemically in combination with long-wave ultraviolet light (UV-A). This treatment is known by the name of PUVA.

8-MOP seems to be a weak allergen as allergic reactions are rarely mentioned in the literature. Fulton & Willis (1978) reported three patients with photoallergy to topically applied 8-MOP. Ljunggren (1977) listed photoallergy to psoralens from contact to parsnip (*Pastinaca sativa*), giant hogweed (*Heracleum mantegazzianum*) and garden parsley (*Petroselium sativum*). Recently there has been a case report of a patient who showed a photoallergic reaction to systemic and topical administration of 8-MOP (Plewig, Hofmann & Braun-Falco, 1978). However, no pure contact allergy against 8-MOP has yet been reported.

## CASE REPORT

A 20-year-old male patient, suffering from alopecia areata of the ophiasis-type for more than 1 year, was treated with topical photochemotherapy to induce regrowth of hair (Weissmann *et al.*, 1978). An 8-MOP solution (Meladinine, Basotherm, Germany) was applied 1 h prior to UV-A irradiation in a high intensity unit (Waldmann 6000, Germany). After the sixth treatment the patient showed a papulovesicular itching dermatitis in the treated areas. Photoallergic dermatitis to the psoralen was suspected. The treatment was discontinued and the dermatitis cleared rapidly after topical application of corticosteroids. Re-exposure to 8-MOP a few days later resulted in a re-occurrence of dermatitis.

## MATERIALS AND METHODS

The 0·15% 8-MOP solution (Meladinine) was applied to two areas on the patient's back. One area was covered with a tape, impermeable to light (Normaflex Plastik Pflaster, Dr Hessle, Vienna,

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Austria). One hour later the other area was exposed to 0·5 J/cm$^2$ UV-A, the dose the patient had received in the initial phase of the treatment.

The ingredients* of the commercially available 8-MOP solution namely adipic acid isopropyl ester, isopropanol and 8-MOP were used for patch testing and photopatch testing (0·1 J/cm$^2$ and 0·2 J/cm$^2$ UV-A) (Bandmann & Fregert, 1973). 8-MOP was applied as pure substance, the solvents in the commercially used concentrations of the 8-MOP solution.

Controls included ten healthy subjects. Readings were done after the removal of the patches and up to 72 h later.

The commercially available 0·15% 8-MOP solution was diluted 1:10, 1:100, 1:1,000, 1:10,000 and tested as described above, with 0·1 and 0·2 J/cm$^2$.

Biopsies were taken from positive patch tests and photopatch tests 48 h after applying the 8-MOP, i.e. 24 h after irradiation with UV-A.

## RESULTS

Twenty-four hours after application of the 0·15% 8-MOP solution positive tests were seen in the patch-test and the photopatch-test areas. There were papulovesicles extending approximately 2 cm beyond the test areas. There was a crescendo reaction up to 72 h. The photopatch test areas reacted slightly more than the patch test areas.

There was no reaction to the solvents.

Control subjects did not react to any ingredient of the 8-MOP solution.

Patch tests were positive to the 1:10 dilution of the 0·15% 8-MOP solution, showed a questionable reaction to the 1:100 dilution and no reaction to the higher dilutions. Open and closed patch test areas reacted alike. Photopatch test areas, however, showed a slightly stronger reaction as compared to the non-irradiated test areas.

Histologically, there were typical signs of contact allergy, namely perivascular inflammation with

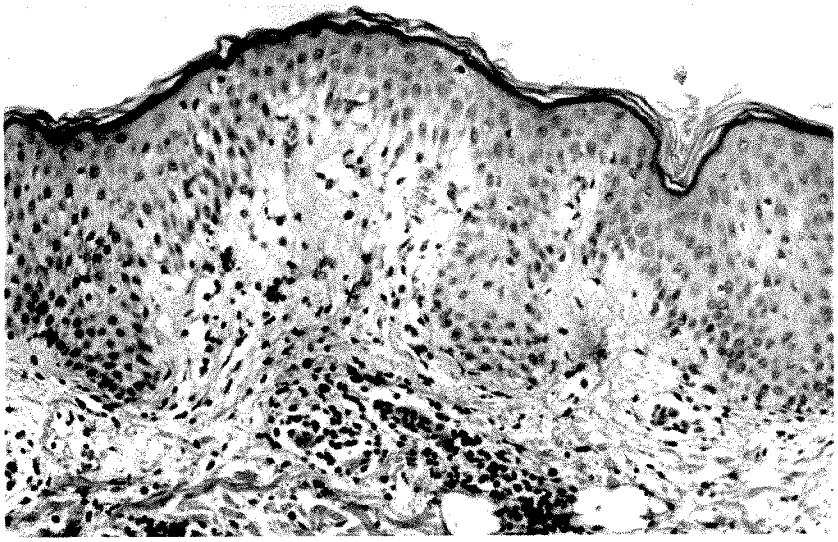

FIGURE 1. 8-MOP contact allergy. Spongiotic dermatitis, perivascular round cell infiltrate, partly directed towards the epidermis (H & E, ×90).

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* Materials kindly supplied by Basotherm, Germany.

round cell infiltrates in the upper and middle dermis, partly directed towards the epidermis, with spongiosis. The corneal layer was intact (Fig. 1). The UV-A-irradiated site was very similar and no sunburn cells could be found.

## DISCUSSION

The diagnosis of the contact allergy to 8-MOP presented in this paper could be proven by means of several tests.

### *Identification of the allergen*

Patch testing with the ingredients of the 8-MOP solution showed a positive reaction only to 8-MOP; therefore 8-MOP could be verified as the responsible agent for the observed dermatitis. Cross reactions to 5-methoxypsoralen and trimethylpsoralen were not investigated.

### *Exclusion of toxic reaction*

By testing ten control patients a toxic reaction to 8-MOP was excluded. The crescendo type of the test reaction as well as the spreading phenomenon support the allergic nature of the reaction.

Histologically there were typical signs of contact allergy as described above.

### *Exclusion of a photoallergic or phototoxic reaction*

By covering the non-UV-A irradiated areas with a tape impermeable to light, light-mediated reactions or a 'masked' photopatch test were excluded (Epstein, 1963).

The patient reacted to the photopatch test of pure 8-MOP and to the 1:10 solution of the commercial product, with a slightly stronger reaction in the light-tested areas. This increased reaction is probably caused by the additional well known phototoxic reaction to 8-MOP.

Theoretically there could be an additional photoallergic reaction together with the phototoxic reaction.

As the histological changes of photoallergic contact dermatitis are indistinguishable from those of allergic contact dermatitis (Ackerman, 1978), and the photoallergic as well as the phototoxic reaction to 8-MOP are caused by UV-A, histology seems unlikely to answer the question if there is an additional photoallergy.

## ACKNOWLEDGMENT

This study was kindly supported by the Deutsche Forschungsgemeinschaft grant Br 147/40.

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## ADDENDUM

Since this paper was accepted for publication a patient with contact allergy to methoxsalen has been described by Saihan, E.M. (1979) *British Medical Journal*, **ii**, 20.

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## *Correspondence*

### Stockinette for dermatological dressings

Sir, I am pleased to say that, after prolonged negotiations, it has been possible to include, in the Second Amendment of the 1979 Drug Tariff, a range of sizes of stockinette so that dermatological patients can have suitable stockinette dressings.

The following sizes of ribbed stockinette may be ordered from 1 October 1979. General practitioners and pharmacists have been warned in advance by means of a Family Practitioner Notice (FPN 234).

The details are shown in Table 1.

TABLE 1.

| Appliance Ribbed surgical tubular stockinette (ribbed stockinette) for use as protective dressings with tar-based and other non-steroid ointments | Size (cm × m) | Basic price (p) per packet |
|---|---|---|
| Arm/leg (child): arm (adult) | 5 × 5 | 55 |
| Arm (OS adult): leg (adult) | 7·5 × 5 | 75 |
| Leg (OS adult) | 10 × 5 | 100 |
| Trunk (child) | 15 × 5 | 150 |
| Trunk (adult) | 20 × 5 | 175 |
| Trunk (OS adult) | 25 × 5 | 200 |

N.B. One 5 m length of the relevant width is sufficient to provide two sets of dressings for a pair of limbs or a trunk. Two full suits for an OS adult are provided from one pack each of the 7·5 cm, 10 cm and 25 cm widths. Two full suits for a standard sized adult are provided from one pack each of the 5 cm, 7·5 cm and 20 cm widths. Two full suits for a child are provided from two packs of the 5 cm widths and one pack of the 15 cm width.

Stockinette is washable for home dressings and a suit can last about 10 days if reapplied daily.

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N.R.Rowell

*British Journal of Dermatology* (1980) **102,** 118.

# *Book Reviews*

**Chemosurgery. Microscopically Controlled Surgery for Skin Cancer.** By FREDERIC E. MOHS (1978) Springfield, Illinois: C. C. Thomas. Pp. 378, 254 illustrations. Price $43.75.

Mohs' technique for the controlled excision of skin cancer has not received as much attention in this country as it deserves. This is not because it has been tried and discarded but because we have been—perhaps unwarrantedly—complacent about the results achieved by conventional methods. The wide availability of Radiotherapy and Plastic Surgery units concentrate expertise on the more difficult examples of malignancies of the skin and the dermatologist does not normally undertake surgery on any but superficial and easily accessible tumours. However, it is obviously very satisfactory to be able to trace and be shown to have removed every strand and thread of a basal cell carcinoma that may have deceptively deep tentacles. The achievement of Frederic Mohs in perfecting and simplifying this technique of histologically controlled serial excision gives him full authority to present the results of many years' experience in his chosen field, in which he is undoubtedly master.

The first part of this profusely illustrated book deals with the method and apparatus required; this is followed by sections dealing with basal and squamous cell lesions in different situations and circumstances, each ending with tables showing the results obtained. The cure rate is certainly impressive for primary lesions with no evidence of metastases. The credit for this cannot all be given to the expertise of one man and his team, even if they are supremely efficient in one particular technique; the logic of the approach commands equal respect.

The second part of the book deals with special areas such as the ear and lip and with the extension of controlled excision to the mouth, cervix and even the larynx. The author also discusses the role of the fresh tissue technique which does not require prior fixation and which greatly reduces the discomfort of the patient and the time taken.

Even if the reader does not, himself, undertake surgery on but the smallest of superficial lesions, he will be stimulated by Mohs' views on the mode of spread of cancer. Having read this chapter he will not, I think, put the book down easily. All dermatologists will be especially interested in the good results achieved in malignant melanomas. Though the figures are still relatively small to make comparisons entirely valid, his results in tumours of levels III and IV, the really difficult ones in which to predict a prognosis, are encouraging.

Though the provision and expense of trained technicians may limit the widespread adoption of the Mohs' procedure in the present economic climate, it is important to have this comprehensive guide available.

D.S.WILKINSON

**Botanical Dermatology. Plants Injurious to the Human Skin.** By J.MITCHELL and A.ROOK (1979) Philadelphia: Lea and Febiger (Distributed in U.K. by Henry Kimpton Ltd, London). Pp 787. Price £30.00.

This remarkable book is encyclopaedic in its coverage of botanical dermatology. The introduction describes the general, historical and clinical aspects and the main subject is then set out in an orderly fashion. The 248 families are arranged in alphabetical order and the 1,400 genera and thousands of species are dealt with in turn. The text is concise and the information concerning each plant is followed by a comprehensive list of references ranging from the current to the historical. The subject matter includes the chemical aspects, dermatitis and also toxic reactions and the whole is leavened by a wealth of interesting details about the plants.

This book is a remarkable achievement and is the distillation of years of study by the authors and we are greatly indebted to them for compiling and publishing this comprehensive text. It will be of value not only to dermatologists but also to industrial medical officers, botanists, horticulturalists and all who are interested in plants and the environment.

E.CRONIN

**Dermatology. Diagnosis and Treatment of Cutaneous Disorders,** 4th edn. By WILLIAM D. STEWART, JULIUS L. DANTO and STUART MADDIN (1978) St Louis: C. V. Mosby Company. Pp. 621, 277 illustrations, 62 col. plates. Price £22.25.

This book began life in 1966 under the title 'Synopsis of Dermatology' and over three editions has been enlarged and improved. It is now re-published under this new title in its 4th edition. The authors point out in their Preface that this is a book on clinical dermatology and is not intended to be a comprehensive textbook. In excluding much that is frankly rare and concentrating on the common and many not so common skin diseases, they have produced a highly readable and up-to-date text on clinical dermatology which will be ideal for those with little dermatological experience, but which also has much to offer the established clinician.

The first two sections of the book, which will be particularly useful to the dermatological beginner, deal with skin conditions classified by body region and with dermatological therapeutics and techniques. The third and main section deals with specific dermatological disease entities. Recent advances in basic scientific knowledge, which have a bearing on the clinical situation, have been included where possible, and treatment of individual conditions—so often poorly described in standard texts—is particularly well covered.

A particularly useful feature of the book is that each section is followed by an up-to-date list of key references—which have each been summarized in a succinct one-sentence form by the authors.

The book is extremely well set out and illustrated, thus making it a pleasure to read. It will rightly find its way into many Medical and Dermatological Libraries, but its high price is likely to preclude many from buying their own individual copy.

R.D.G.PEACHEY

**Atlas of Rectoscopy and Colonoscopy.** By P. OTTO and K. EWE (1979) Berlin: Springer-Verlag. Pp. 110, 124 col. illustrations. Price DM 98.

The first part of this small book discusses the technical aspects of digital rectal examination, proctoscopy and colonoscopy, and the second part consists of brief summaries and colour pictures of the various conditions which may be diagnosed by these techniques.

Dermatologists may find the technical section helpful, but in the second part of the book, the colour pictures are of variable quality, and some of the pathology is questionable. The sections devoted to the anus and peri-anal skin are particularly disappointing. Paget's disease and lichen sclerosus et atrophicus are not mentioned, and several statements seem to have lost in the translation. Thus we read that for condyloma acuminata 'a side-effect of gonorrhoe (sic) is certainly possible', villous adenoma produces 'frawn-like emminences', and 'soor mycoses' cause eczema. A minor irritation is the authors' reluctance to use absolute units, so that a rectal prolapse, for example, is described as being 'up to a child's head in size'.

J.L.BURTON

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# *News and Notices*

## Nederlandse Vereniging voor Dermatologie en Venereologie

The next meeting of the association will be held on 23 February 1980, in the Binnengasthuis (the Dermatological Department of the Municipal University, head Prof. Dr R. Cormane).

On 26 April Prof. Dr E. van Dijk, head of the Dermatological Department of the Free University in Amsterdam, will receive the Nederlandse Vereniging voor Dermatologie en Venereologie and guests.

Further information may be obtained from S. M. C. Molhuysen van der Walle, Guido Gezellestraat 6 1077 WP Amsterdam, The Netherlands.

## The American-International Conference on Clinical Dermatology

This conference will be held at the RAI-Congress Center, Amsterdam, The Netherlands, 15–18 June 1981. The programme will be presented by a large, internationally recognized faculty and will emphasize recent developments in the management of major skin disorders of importance to all clinical dermatologists.

To obtain a preliminary programme or for further information, please write to: Dr Richard L. Dobson, Department of Dermatology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29403, U.S.A.

## Appointments

### Devon Area Health Authority

Dr Andrew Peter Warin has been appointed Consultant Dermatologist at the Royal Devon and Exeter Hospital (Wonford), Exeter. Dr Warin graduated M.B., B.S., in 1967 (Guy's Hospital) and became M.R.C.P. (U.K.) in 1970. His pre-specialist training appointments were at Guy's Hospital, and in 1971 he became Registrar in Dermatology at St Thomas' Hospital, and in 1973 Senior Registrar at the London Hospital. In 1974 he transferred to St John's Hospital as Senior Registrar and then spent a year in Shiraz, Iran as associate professor in Dermatology at the Pahlavi University Medical School. In April 1976, he was appointed Consultant Dermatologist at St John's Hospital and Honorary Senior Lecturer at the Institute of Dermatology.

He has published papers on many subjects including the following: the glucagonoma syndrome, disseminated gonorrhoea, the effects of methotrexate on the liver, photochemotherapy of psoriasis and mycosis fungoides, and hereditary angioedema. He has studied the role of prostaglandins in the ultraviolet erythemas under the direction of Professor M.W. Greaves.

He has been responsible for the establishment of the PUVA unit at St John's Hospital, and in 1978 ran a 3-day conference on photochemotherapy. He is currently serving on the council of the British Association of Dermatologists, the committee of Dermatology of the Royal College of Physicians and is Honorary Editor of the Dermatology section of the Royal Society of Medicine. He has been the Honorary secretary of the St John's Hospital Dermatological Society and is Assistant Editor of Clinical and Experimental Dermatology.

### Survey Area Health Authority

Colonel Alan Jarrams has been appointed Consultant Dermatologist to the Surrey Area Health Authority based at St Peter's Hospital, Chertsey. He graduated M.B. Ch.B. (Birmingham) in 1948 and after house appointments did National Service in the RAMC, which he rejoined after 12 years in General Practice in Newport, Monmouthshire. Dermatological training followed in Military Hospitals in Aldershot and London and in St John's Hospital for the Diseases of the Skin.

He has served in Germany, Singapore and Hong Kong studying the prevalence of leprosy in Gurkha troops and nail growth rates in various ethnic groups of British Servicemen.

## Annual Meeting of the British Association of Dermatologists—Oxford 1980

This will be held in Oxford on 9–12 July 1980 under the Presidency of Dr D.S.Wilkinson. The business meeting will be held on Wednesday 9 July at 3.30 p.m. and all Thursday and Friday morning will be devoted to scientific papers. The clinical meeting will be held on Saturday morning, 12 July. All the meetings will take place in the John Radcliffe Hospital, Oxford. The social and sporting activities will be held as usual.

Papers are invited and should be sent in the form of a summary of approximately 200 words (six copies) to the Hon. Secretary, Dr K.V.Sanderson, The British Association of Dermatologists, 7 John Street, London WC1N 2ES before 15 March 1980. If there is more than one author, please underline the name of the presenter.

Scientific exhibits are particularly welcomed. A title and a brief outline should also be sent in the first instance to the Honorary Secretary. Further details can be obtained from Dr T.J.Ryan, The Slade Hospital, Oxford OX3 7JH.

Accommodation has been reserved at Keble and Pembroke colleges. Those wishing to book hotel accommodation are strongly advised to make early arrangements with Thos. Cook & Sons Ltd or their local Cook's/Wagons Lits agents, who have arranged some block bookings. Parking and dining facilities are also very restricted in Oxford in July and early booking of the latter is advisable. The 'Cherwell Guide to Oxford' gives much information and can be purchased for 75p from the Oxford Student Publications Ltd, 9a Saint Nicholas Street, Oxford OX1 23D.

Further information may be obtained from the Honorary Secretary and Dr Dawber, the local secretary for the meeting, will be pleased to answer any queries concerning the meeting itself.

## The British Society for Dermatopathology

A meeting of the British Society for Dermatopathology is to be held prior to the meeting of the British Association of Dermatologists on Wednesday, 9 July 1980. This educational meeting will cover various aspects of dermatopathology and in the afternoon Professor A. Bernard Ackerman from New York University will be one of the guest speakers.

The meeting will be held in the John Radcliffe Hospital, Oxford, starting at 10.00 a.m., and the registration fee is £10.00 for non-members of the Society.

Further details concerning the programme will be available from Dr Neil Smith, Secretary, British Society for Dermatopathology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

## British Contact Dermatitis Group

An inaugural meeting of this new Group will be held at Oxford on the late afternoon of Tuesday, 8 July 1980. Those interested in active participation should contact Dr R.J.G.Rycroft, St John's Hospital for Diseases of the Skin, Lisle Street, London WC2H 7BJ or Dr J.D.Wilkinson, St Thomas' Hospital, London SE1.

## ESSENTIAL DERMATOLOGY BOOKS

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| milligram(s) ($10^{-3}$ g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$ g) | μg | cubic millimetre(s) | mm$^3$ | micrometer(s) | μm |
| nanogram(s) ($10^{-}$ g) | ng | millimetre(s) | mm | per cent | % |
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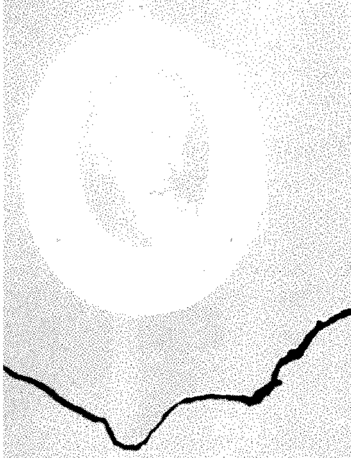

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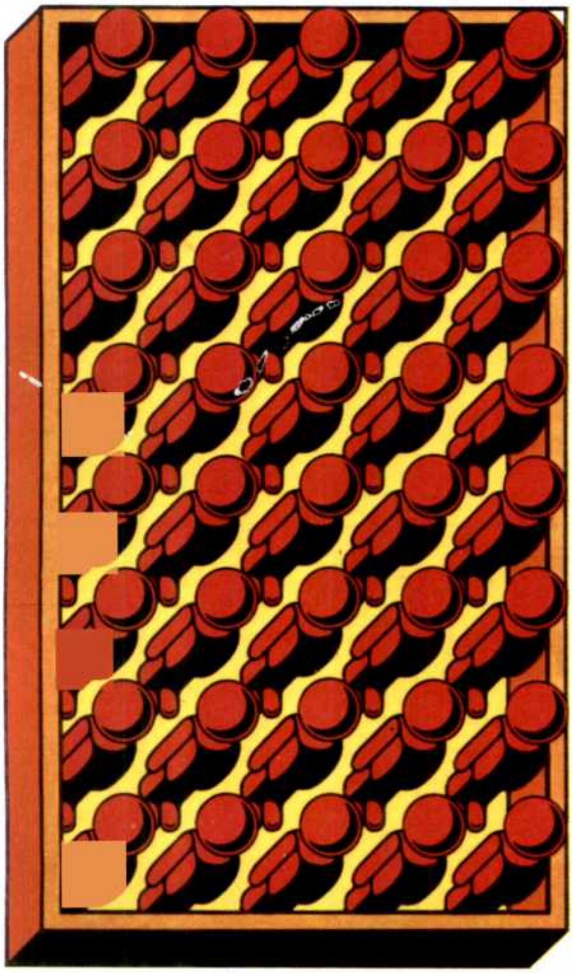

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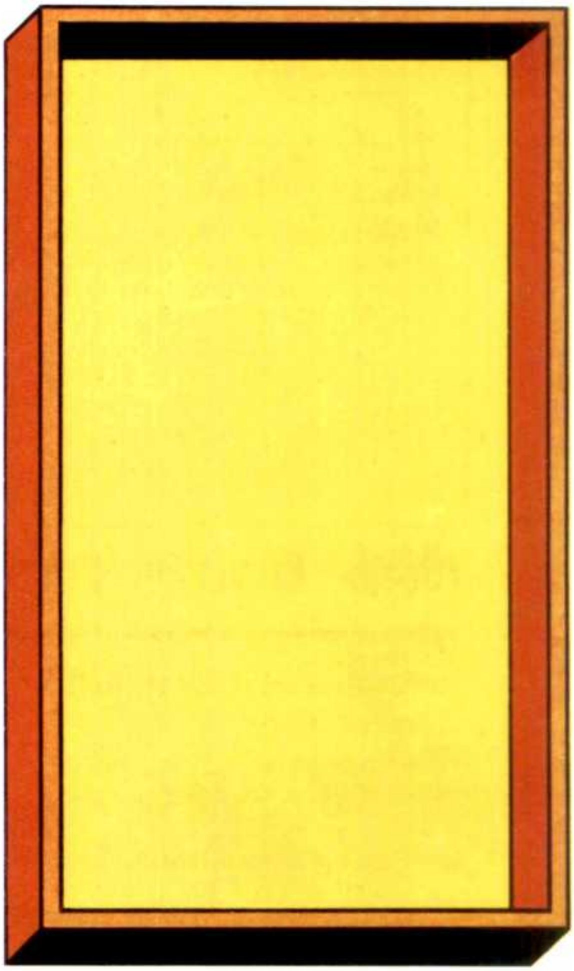

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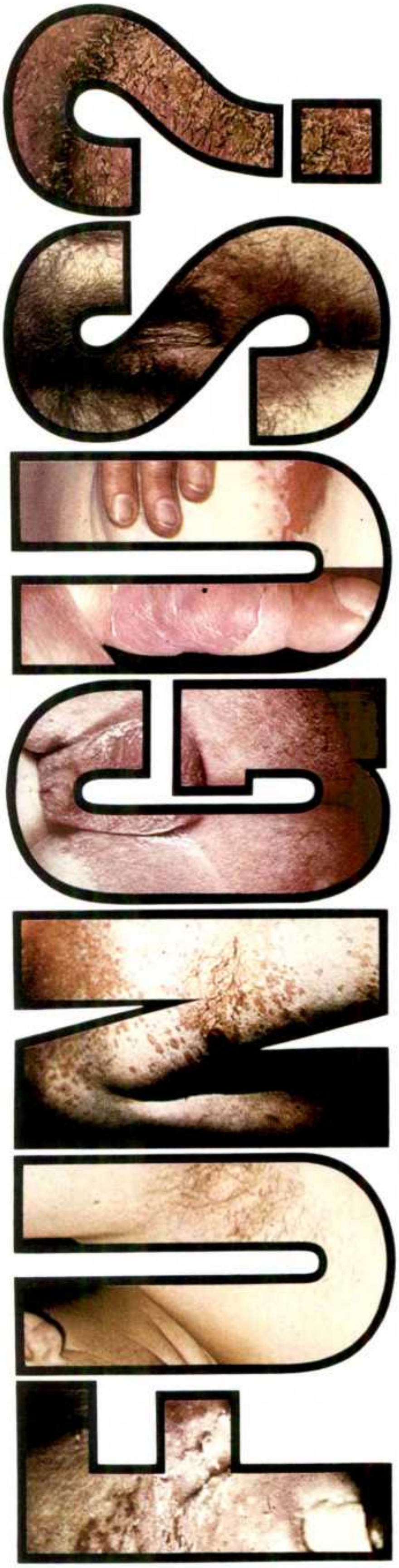

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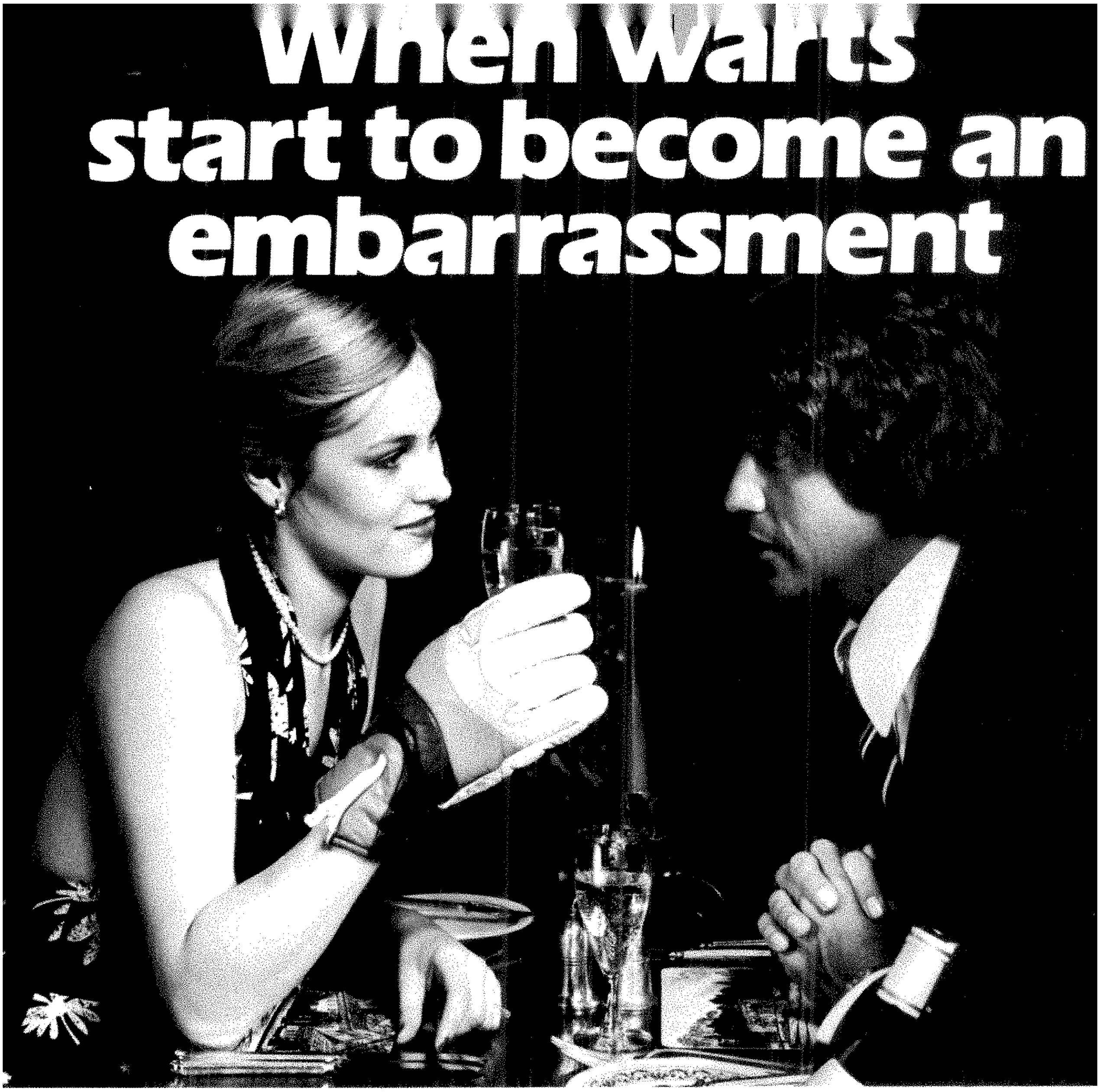

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*Directions for Use:* Paint the wart, cover with a waterproof plaster and leave for 24 hours. Remove collodion by rubbing with a piece of pumice stone and repeat application.
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(lactic acid BP, salicylic acid BP)

*British Journal of Dermatology* (1980) **102**, 123.

***Clinical and Laboratory Investigations***

# The stratum granulosum: a contractile system

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## SUMMARY

Sheets of stratum granulosum of glycerinated cow hoof epidermis showed immediate contraction on exposure to ATP in the presence of calcium. Other layers of the epidermis were unresponsive. Contraction of the stratum granulosum could be induced also by dopamine, but not by a variety of other pharmacological agents. It could be blocked by EGTA, lidocaine, or high concentrations of potassium. It is postulated that the keratohyalin granule is a contractile protein uniquely responsible for the changes observed.

As the outermost layer of living cells of the body, the stratum granulosum is unique, not only in its position, but in its cellular architecture. Its cells show distinctive granules which for over a hundred years have eluded attempts to determine their significance (Ugel, 1977). Sighted by Aufhammer (1869) in palmar skin and perceived by Langerhans (1873) to cover the entire body, the stratum granulosum has long been viewed simply as the final stage of differentiation of the keratinocyte. Yet the functional nature of its granules remains as obscure as when they were first named keratohyalin by Waldeyer (1882).

The present study reveals for the first time that the stratum granulosum is a unique contractile system. This has given an understanding of the function of this outermost sheath and in turn its distinctive granules.

## MATERIALS AND METHODS

Experiments were done on both pigmented and non-pigmented normal glabrous skin taken from fresh autopsy specimens of cow hoof just posterior to the plate (Ugel & Idler, 1970). Long, narrow (30 × 3 mm) strips of full epidermis but minimal corium were placed in 50% glycerol distilled water v/v prechilled to 0°C. After 24 h the bottled specimens were further cooled to −20°C and kept stored at this temperature for a maximum of 6 months. Blocks of unthawed tissue (2 × 3 mm base) were mounted in ice, corium side up, and serial 14 μm frozen sections were cut parallel to the stratum

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corneum surface. Sectioning was done at −25°C on a Slee HR Mark II cryostat or an International-Harris (CTD Model) cryostat. Using a finely pointed wooden tip the sections were transferred directly to the test solutions at room temperature. Direct and photographic observations on conformational change were made directly with the unaided eye as well as under a dissecting microscope (up to × 30).

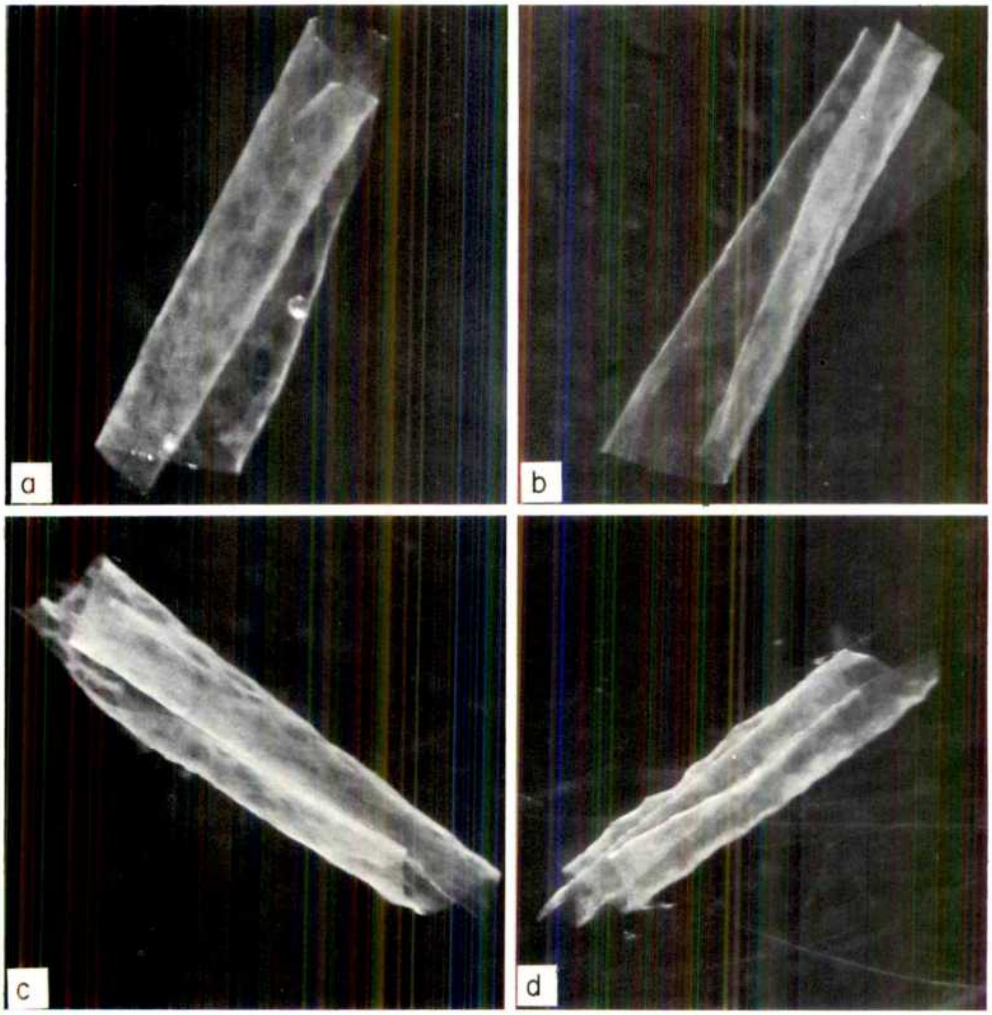

FIGURE 1. Classification of degrees of ATP-induced contraction (rolling, furling) of stratum granulosum sections as employed in Tables 1–10. (a) 1+; (b) 2+; (c) 3+; (d) 4+.

Classifications were made according to general patterns shown in Figs 1 and 2. Histological identification of section composition was then made on immediately fixed and stained (H & E) specimens.

The following special chemicals (Sigma, St Louis, Mo.) were studied: adenosine 5′-diphosphate sodium salt (ADP) 95–99% (equine muscle), adenosine 5′-triphosphate (ATP), dibarium, dicalcium (90–95%), diethanolamine, dipotassium (95–98%), disodium crystalline low calcium (99–100%), Mg (95–98%), tris (hydroxymethyl aminomethane) (sodium < 0·1%) salts prepared from equine muscle and essentially free of vanadium; $\gamma$ amino-n-butyric acid (GABA); 1-cysteine (free base), deoxyepinephrine (epinine, N-methyldopamine); L-$\beta$-3-4 dihydroxyphenylalanine (Dopa); 1-epinephrine bitartrate; ethylenediamine tetraacetic acid (EDTA); ethyleneglycol-bis-(B-amino-ethyl ether) N,N′-tetraacetic acid (EGTA); histamine dihydrochloride; 5-hydroxytryptamine creatinine sulfate (serotonin); 3-hydroxytyramine HCl (dopamine); inosine-5′-triphosphate (sodium salt, 95–97% equine mixture); lidocaine free base (Xylocaine); L-norepinephrine bitartrate (L-arterenol bitartrate) crystalline, 98%. Also studied were Zwitterionic buffers (Boehringer-Mannheim Biochemicals): MES (pKa 5·96, 2-(N-morpholino) ethanesulfonic acid); PIPES (pKa 6·66, piperazine-N, N′-bis (2-ethanesulfonic acid); MOPS (pKa 7·01, N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid); HEPES (pKa 7·31, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid); BICINE (pKa 8·04, N,N-Bis (2-hydroxyethyl) glycine).

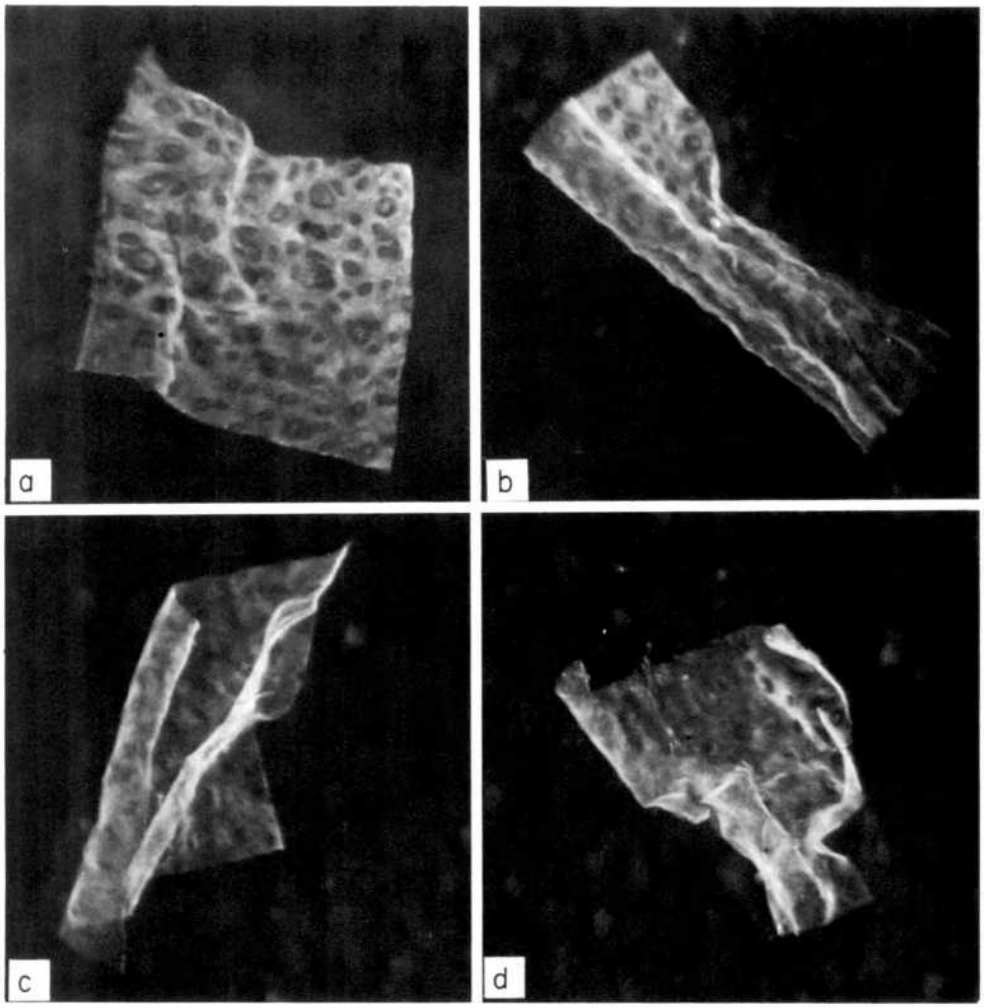

FIGURE 2. Classification of degrees of ATP-induced contraction (pleating, wrinkling, twisting, curling) of stratum granulosum sections as employed in Tables 1–10. (a) 1 +; (b) 2 +; (c) 3 +; (d) 4 +.

The majority of experiments were done in the following contraction medium at 21°C: 170 mM NaCl (sodium chloride), 10 mM MOPS (morpholinopropane sulphonic acid), 2 mM $CaCl_2$ (calcium chloride), 2 mM $MgCl_2$ (magnesium chloride), 1 mM L-cysteine (free base) buffered at pH 7·2.

Water used was ion-free, dimineralized, distilled (Water Purifier LO-3 with ultrahigh capacity Inorganic Cartridge, Mega-Pure Still (Corning)). Additional buffered solutions studied included: Gomori Tris-maleate, Michaelis' isotonic Veronal-sodium acetate, and Sörenson 0·067 M phosphate (isotonic) (Gabe, 1976).

## RESULTS

The data are presented in tabular form with Figs 1 and 2 illustrating the specific rating of contractile responses recorded. In essence, ATP salts in a concentration of 1·0 mM or greater regularly induced virtually immediate contractions in 14 $\mu$m sheets containing a significant amount of stratum granulosum (Tables 1 and 2), and this effect was increased in the presence of calcium. The phenomenon was not seen in sections of stratum spinosum or stratum corneum.

TABLE 1. Effect of concentration of ATP. Mg-ATP in 2 mM $CaCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2

| Concentration (mM) | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| | Number of sections | | | | |
| 0 | 25 | 3 | 1 | 0 | 0 |
| 0·5 | 24 | 2 | 2 | 1 | 0 |
| 1 | 24 | 2 | 0 | 5 | 2 |
| 2 | 32 | 5 | 1 | 3 | 2 |
| 5 | 23 | 5 | 4 | 3 | 2 |
| 10 | 23 | 3 | 3 | 2 | 2 |

TABLE 2. Effect of addition of ions. Medium: 2 mM NaATP, 10 mM MOPS, 170 mM NaCl, pH 7·2

| Ion | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| | Number of sections | | | | |
| Control | 24 | 2 | 3 | 2 | 0 |
| $CaCl_2$ (2 mM) | 19 | 6 | 4 | 4 | 7 |
| $MgCl_2$ (2 mM) | 39 | 5 | 3 | 2 | 2 |
| Cysteine (1 mM) | 21 | 3 | 1 | 0 | 0 |
| Glucose (4 mM) | 27 | 6 | 2 | 2 | 0 |
| $CaCl_2 + MgCl_2$ | 16 | 8 | 2 | 3 | 3 |
| $CaCl_2$ + cysteine | 30 | 11 | 5 | 3 | 3 |
| $CaCl_2 + MgCl_2$ + cysteine | 18 | 5 | 7 | 3 | 3 |

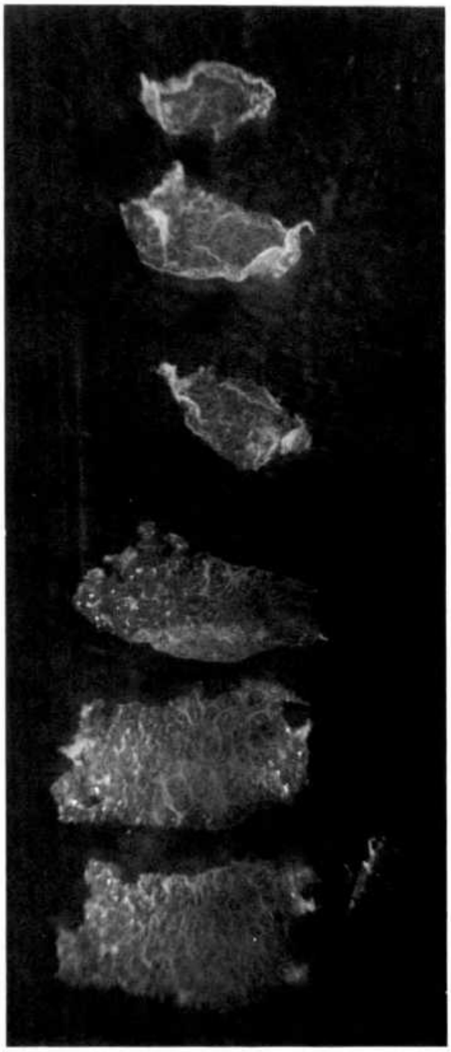

FIGURE 3. ATP-induced contractions in serial horizontal sections of glycerinated cow hoof epidermis. The upper three sections showing contraction are cuts through the stratum granulosum. The lower three showing no contraction and a silhouette × 4 larger are in the stratum spinosum. Not shown are the stratum corneum sections above these which likewise remained flat, showing no contractile response. Technique: serial sections (14 μm) parallel to skin surface, photographed *in situ*, 5 min after immersion in 2 mM Mg ATP in buffered saline (× 15).

Progressive horizontal sectioning of the epidermis from the dermis upward showed no response to ATP until the stratum granulosum was reached (Fig. 3). This response was again lost when the stratum corneum was reached. In specimens in which the granulosum comprised but a part of the section only the granulosum portion contracted. In a few instances in which the granulosum was only in the central part of the section remarkable butterfly like forms appeared, the non-contractile segments forming wing-like appendages coming off the central contracted body.

Regular cross sections failed to show any gross contractile change. However, sections cut at such a

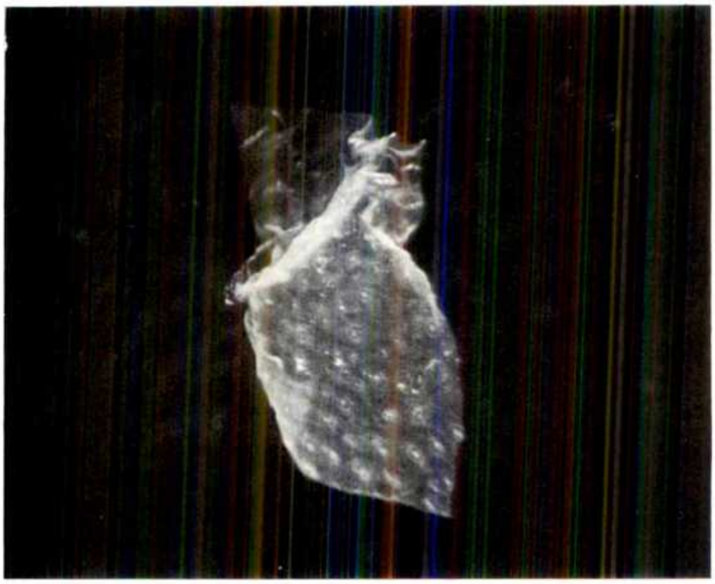

FIGURE 4. ATP-induced contraction localized to stratum granulosum in central zone, obtained by oblique cross section of cow hoof epidermis. Triangular top portion is stratum corneum and lower part of section which is also flat is stratum spinosum. Glycerinated specimen (14 $\mu$m) 3 min after immersion in 2 mM Mg ATP in buffered saline solution (× 25).

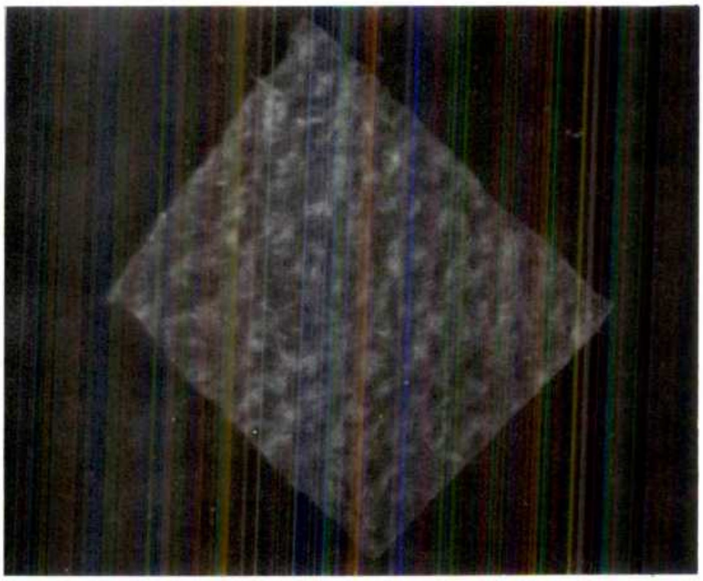

FIGURE 5. Section of stratum granulosum in control buffer solution shows no contraction (2 mM calcium chloride, 1 mM cysteine, 2 mM magnesium chloride in physiological saline solution buffered to pH 7·2 with 10 mM MOPS).

tangential angle as to increase the proportion of stratum granulosum showed contraction limited precisely to this stratum (Fig. 4). At other times this presence manifested itself as a hinge fold, the stratum corneum folding over the stratum spinosum with the stratum granulosum as the hinge. With perfectly aligned horizontal sections the usual response was a roll (Fig. 1) of a varying number of coils and tightness. Scalloping and wave-like ridges could be seen along the border. Other sections showed pleating, twisting, folding and wave forms, again correlating with the variable presence of the stratum granulosum (Fig. 1). All of these contracted specimens resisted attempts at flattening or straightening, unlike the loose rolls seen in the control. Again, in contrast to the free floating flat control sections, these specimens often sank to the bottom of the dish.

TABLE 3. Effect of various salts of ATP. 2 mM ATP salts in 2 mM $CaCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| ATP salt | Number of sections | | | | |
| Ba | 32 | 4 | 2 | 0 | 0 |
| Ca | 32 | 3 | 3 | 2 | 0 |
| Diethanolamine | 28 | 5 | 3 | 2 | 0 |
| K | 23 | 5 | 2 | 2 | 0 |
| Mg | 27 | 4 | 4 | 2 | 4 |
| Na | 22 | 7 | 1 | 3 | 2 |
| Tris | 22 | 6 | 5 | 1 | 0 |

TABLE 4. Effect of various buffers. 2 mM NaATP, all buffers at pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Buffer | Number of sections | | | | |
| HEPES (10 mM in saline) | 23 | 8 | 1 | 2 | 2 |
| MOPS (10 mM in saline) | 27 | 4 | 4 | 3 | 3 |
| Phosphate (Sorenson) | 28 | 5 | 1 | 1 | 0 |
| Tris-Maleate (Gomori) | 24 | 2 | 2 | 3 | 0 |
| Veronal (Michaelis) | 39 | 6 | 2 | 3 | 2 |

TABLE 5. Effect of pH. 2 mM NaATP, 2 mM $CaCl_2$, 1 mM cysteine, Veronal-Acetate (Michaelis) buffer

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| pH | Number of sections | | | | |
| 4·0 | 26 | 4 | 2 | 5 | 4 |
| 5·0 | 28 | 8 | 4 | 3 | 3 |
| 6·0 | 25 | 6 | 2 | 6 | 5 |
| 7·0 | 30 | 4 | 2 | 1 | 3 |
| 8·0 | 40 | 3 | 4 | 0 | 0 |
| 9·0 | 36 | 5 | 1 | 0 | 0 |

TABLE 6. Effect of tonicity. 2 mM NaATP in 2 mM calcium, 2 mM $MgCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| NaCl (nM) | Number of sections | | | | |
| 120 | 45 | 3 | 1 | 0 | 0 |
| 170 | 25 | 6 | 3 | 4 | 4 |
| 220 | 49 | 0 | 0 | 0 | 0 |

TABLE 7. Effect of temperature. 2 mM NaATP, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Temperature (°C) | Number of sections | | | | |
| 0 | 35 | 2 | 0 | 0 | 0 |
| 21 | 15 | 6 | 7 | 5 | 7 |
| 37 | 30 | 3 | 2 | 6 | 6 |

The sodium and magnesium salts of ATP proved to be the most active (Table 3), and could be used in a variety of buffers (Table 4), at neutral and acid pH (Table 5). Significant alterations in the tonicity of the solution abolished the contractile response (Table 6). The addition of calcium had a dramatic effect (Table 2) and lowering the temperature of the solution to 0°C abolished the response (Table 7). Fourteen micron thick sections proved to be the best (Table 8). Thinner sections were difficult to handle in a routine fashion, and thicker sections showed little or no gross evidence of contraction.

In the absence of ATP, the response could be triggered only by INT, dopamine or methyl dopamine (Table 9). Inhibition of the response occurred in the presence of a number of agents (Table 10), the dopamine contractions being inhibited selectively by the antagonist GABA.

## DISCUSSION

Our demonstration of a specific band of contractility within the epidermis evolved from the employment of Szent-Gyorgyi's (1949) technique of preparing a glycerinated muscle model. He discovered that it was possible to induce contraction of glycerinated muscle specimens by the simple addition of

TABLE 8. Effect of thickness of section. 2 mM NaATP, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2. Control: same with no ATP

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Thickness (μm) | Number of sections | | | | |
| 7 Control | 41 | 6 | 4 | 4 | 3 |
| 7 ATP | 25 | 7 | 15 | 8 | 7 |
| 14 Control | 34 | 5 | 2 | 0 | 0 |
| 14 ATP | 14 | 4 | 6 | 6 | 6 |
| 21 Control | 35 | 3 | 0 | 0 | 0 |
| 21 ATP | 32 | 2 | 0 | 1 | 1 |
| 28 Control | 26 | 3 | 0 | 0 | 0 |
| 28 ATP | 27 | 4 | 0 | 0 | 0 |

TABLE 9. Effect of drugs. Medium: 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Drug (2 mM) | Number of sections | | | | |
| Control | 24 | 9 | 9 | 0 | 0 |
| ATP | 23 | 7 | 1 | 6 | 5 |
| ADP | 30 | 3 | 0 | 0 | 0 |
| Dopa | 44 | 0 | 0 | 0 | 0 |
| Dopamine | 27 | 4 | 2 | 2 | 1 |
| Epinephrine | 32 | 2 | 0 | 0 | 0 |
| Histamine | 38 | 5 | 3 | 0 | 0 |
| ITP | 19 | 9 | 1 | 7 | 6 |
| Methyl dopamine | 42 | 4 | 2 | 1 | 2 |
| Nor-epinephrine | 37 | 5 | 0 | 0 | 0 |
| Serotonin | 28 | 2 | 0 | 0 | 0 |

TABLE 10. Effect of inhibitors. Medium: 2 mM NaATP, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM $NaCl_2$, pH 7·2

| | Degrees of contraction | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Compound (2 mM) | Number of sections | | | | |
| ATP control | 19 | 6 | 4 | 4 | 7 |
| EDTA control | 21 | 3 | 0 | 0 | 0 |
| EDTA+ATP | 38 | 2 | 0 | 0 | 0 |
| EDTA+dopamine | 35 | 5 | 0 | 0 | 0 |
| GABA control | 34 | 4 | 0 | 0 | 0 |
| GABA+ATP | 35 | 2 | 0 | 4 | 3 |
| GABA+dopamine | 34 | 4 | 0 | 0 | 0 |
| KCl* control | 34 | 0 | 0 | 0 | 0 |
| KCl+ATP | 36 | 1 | 0 | 0 | 0 |
| KCl+dopamine | 50 | 0 | 0 | 0 | 0 |
| Xylocaine control | 31 | 2 | 2 | 0 | 0 |
| Xylocaine+ATP | 38 | 0 | 0 | 0 | 0 |
| Xylocaine+dopamine | 39 | 1 | 0 | 0 | 0 |

* 80 mM.

ATP. In his model, the enzymatic hydrolysis of the ATP provided chemical energy which was translated into visible mechanical contraction by the contractile proteins which remained intact within the cell. His entire specimen was contractile. By contrast it was necessary for us to discover and cut out the contractile zone. This was accomplished by frozen sections cut parallel to the epidermal surface and the zone proved to be the stratum granulosum. The cow hoof was found to be the best source since it has the widest stratum granulosum, in many areas over twenty cells wide. However, the same phenomenon was also observed in other species, e.g. man, guinea-pig, horse and sheep.

Unlike muscle cells which have a simple longitudinal axis of contraction and shortening, the contractile zone of the epidermis is irregular, curved, wavy and involves variable planes. Hence the multiplicity of force vectors and an unavoidable degree of unhomogeneity in specimens result in the many contractile forms observed, at times showing mammillated, haustral, trough, or puckered configurations. Although the surface area silhouette contracted (Fig. 3) there was no simple shortening as seen in muscle.

The technique of preparing frozen sections calls for skill in alignment of the epidermal blocks, a task rendered easier by using pigmented tissue. There is no standard number of potentially contractile sections obtainable from any block so the tabular data cannot be analyzed as precisely as might be desired. Furthermore, washing the specimens in buffer greatly reduces their subsequent contractile force. Apparently the immersion may remove some of the matrix or contractile proteins since, unlike intact isolated muscle cells (Small, 1977), these epidermal cell walls are transected. Our technique furthermore calls for blocking in ice, the specimens mounted in Cryofoam or•O.C.T. Compound being less satisfactory.

A wide variety of pharmacological agents were tested on this new contractile system. It responded only to dopamine and its analogue N-methyl dopamine. This suggests the presence of a dopamine receptor on the granular cell and indeed at least two types of dopamine receptors have been described for the cells elsewhere (Kebabian & Calne, 1979). Interestingly, the response was specifically blocked by the dopamine antagonist GABA. It is notable that the other catecholamines and histamine were not active.

The studies done underscore the need for the presence of an active ATPase. This enzyme was first shown in the granular cell layer by Jarrett & Riley (1963), but its significance remained unknown until now. Our studies would indicate the ATPase to be a Ca-, Mg-dependent type seen in muscle and not an Na-, K-dependent ATPase as seen in association with membrane phenomena. The presence of the calcium–magnesium chelator EDTA or of its analogue calcium chelator EGTA prevented contraction. Thus, preferable buffers included those with insignificant binding of the essential polyvalent calcium ion, e.g. MOPS. Lidocaine, known to inhibit contraction in smooth muscle (Small, 1977), also inhibited the stratum granulosum response. Other known inhibitors of the muscle model contractions (Hasselbach & Weber, 1955), such as zinc chloride 2 mM, sodium fluoride 4 mM, also inhibited these epidermal contractions. Adding formalin to the glycerol preservative completely prevented any contractile response. The enzymatic nature of the contraction was further supported by the absence of response at 0°C. There was a clear association of the degree of ATPase staining and contractility in the stratum granulosum of the guinea-pig foot pad. Finally, the phenomenon did not occur in specimens cut from tissue directly frozen in the absence of glycerol.

The analogy of this epidermal contractile response with that of muscle (Bendall, 1969) is evident. Both have contractile machinery yet histologically there is no resemblance. Furthermore, the potassium ion which favours muscle activity blocks the activity of the granular cells as seen in Sorenson's buffer or on direct addition in high concentration. The thread of resemblance between the two cell systems would appear to be actin. It is a contractile protein known now to be in virtually all eukaryotic cells, including keratinocytes (McGuire, Lazarides & DiPasquale, 1977). In non-muscle cells it appears as an unpolymerized globular form (Actin G) as well as a filamentous form (Actin F) in bundles of parallel microfilaments. Our experiments would suggest that keratohyalin may well be amorphous unpolymerized actin masses which in the presence of ATP become filamentous and actively contractile.

The granular cell should thus be viewed as a highly specialized epidermal cell which has a unique function of contracting. In aggregate, it forms a true myoepithelial layer, undoubtedly related to appendageal myoepithelium in which contractions were directly visualized *in vivo* by Hurley & Shelley (1954).

## ACKNOWLEDGMENTS

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# Detection of basement membrane zone antigens and C3 fragments in the blister fluid of bullous pemphigoid

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## SUMMARY

In polyacrylamide gel gradient electrophoresis, serum and blister fluid of patients with bullous pemphigoid showed a nearly identical protein distribution pattern except for one more concentrated fraction in the blister fluid. This fraction could be isolated by several gel chromatographic steps and then be characterized as a protein with a molecular weight of 240,000 (BFP 240,000) consisting of several subunits. Two of these subunits could be identified as C3 fragments and two other ones as basement membrane zone antigens (BMZ-Ag) by means of a modified Laurell technique. The molecular weight of the BFP 240,000 may be small enough to allow it to penetrate through the vessel wall into the blood circulation where, with the BMZ-antibodies (BMZ-Ab), BMZ-Ag-BMZ-Ab-complexes can be formed. The occurrence of such immune complexes in the serum has been shown in a previous paper.

Bullous pemphigoid represents a humoral autoimmune disease. Freely circulating basement membrane zone antibodies (BMZ-Ab), mostly of the IgG type, have been detected in the serum (Jordan *et al.*, 1967; Beutner, Chorzelski & Jordon, 1970; Chorzelski *et al.*, 1968). Furthermore immune complexes have been found (Jordon & McDuffie, 1976; Braun-Falco, Männel & Scherer, 1977; Tappeiner *et al.*, 1977). In a previous paper we showed that these immune complexes consisted partly of BMZ-Ag-BMZ-Ab complexes (Herrmann *et al.*, 1978). It was the aim of the present study to demonstrate that BMZ-Ag which was not bound to immune complexes occurs in the blister fluid of patients with bullous pemphigoid.

## MATERIALS AND METHODS

### *Patients*

There were five patients in acute exacerbation with massive formation of subepidermal blisters. The direct immunofluorescence method showed deposition of BMZ-Ab (IgG). In some cases freely

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circulating BMZ-Ab could be detected by indirect immunofluorescence, whereas all patients responded well to combined prednisolone-azathioprine therapy.

### *Materials*

Blister fluid and blood were aspirated in a sterile way and allowed to clot at room temperature for 60 min, were centrifuged at 5,000 *g* for 30 min and afterwards frozen at −20°C. The chemicals for polyacrylamide gel electrophoresis, catalase and bovine serum albumin (BSA) were obtained from Serva, Heidelberg, FRG. DEAE-Sephadex A-50 and CM-Cellulose were from Pharmacia Fine Chemicals, Uppsala, Sweden and Biogel A-0·5 m was purchased from Bio-Rad Laboratories, U.S.A. The antisera (see Table 1) and agarose were from Behringwerke AG, Marburg, F.R.G. The FITC-labelled anti-human IgG (rabbit) was obtained from Staatliches Institut für Immunpräparate und Nährmedien, Berlin-Weißensee, G.D.R.

### *Methods*

*Polyacrylamide (PAA)-gel gradient electrophoresis.* This was carried out according to Kopperschläger *et al.* (1969). The linear gradient was performed from 3% to 16% PAA in Tris/HCl gel buffer system (pH 8·4), 4 mA/tube. The protein was stained as described by Diezel, Kopperschläger & Hofmann (1972). The PAA-gel electrophoresis (7·5% PAA) in the presence of 0·1% sodium dodecylsulphate (SDS) was conducted according to Shapiro, Vinuela & Maitel (1967). Then the gel cylinder was put on a glass plate and embedded by a mixture of 2% agarose and 2% PAA. After finishing the polymerization procedure a 2·5% agarose (0·05 mol/l barbital/HCl buffer, pH 8·4) containing anti-C3/C3c serum (10%) and BMZ-Ab serum (12%) of patients with bullous pemphigoid (titre 1:160), respectively, was added to this layer. The electrophoretic analysis was carried out in the second direction in agarose gel containing these antisera at 100 V for 8 h.

*Immunological analyses.* Double immunodiffusion was carried out as described by Ouchterlony (1967). The agarose gel concentration was 2·0% in 0·05 mol/l barbital acetate buffer (pH 8·4). After the formation of the precipitation line, the agarose plate was washed with 0·1 mol/l NaCl solution for 2 days and with distilled water for an additional day. The agarose was dried with filter paper and stained for protein by means of a 0·25% solution of Coomassie brilliant blue G 250 (Diezel *et al.*, 1972).

The two dimensional immunoelectrophoreses were performed by following the principles of the Laurell technique (Laurell, 1965) with the modification that in the first direction a SDS-gel electrophoresis was accomplished.

*Immunofluorescence: indirect method.* Immunofluorescence microscopy investigations were undertaken by the indirect method as described by Coons & Kaplan (1950) and by Beutner, Rhodes & Holborow (1967). Cryostat sections of rabbit tongue were used as antigenic substrate. The FITC-labelled anti-human IgG (rabbit) was used in a working dilution of 1:30. The fluorescence microscope Fluoval 30-6-546 was from VEB Carl Zeiß Jena with epi-illumination technique.

## RESULTS

### *Detection of free BMZ-Ab in the blister fluid*

In the blister fluid free BMZ-Ab of the IgG type up to a dilution of 1:40 could be detected by means of indirect immunofluorescence.

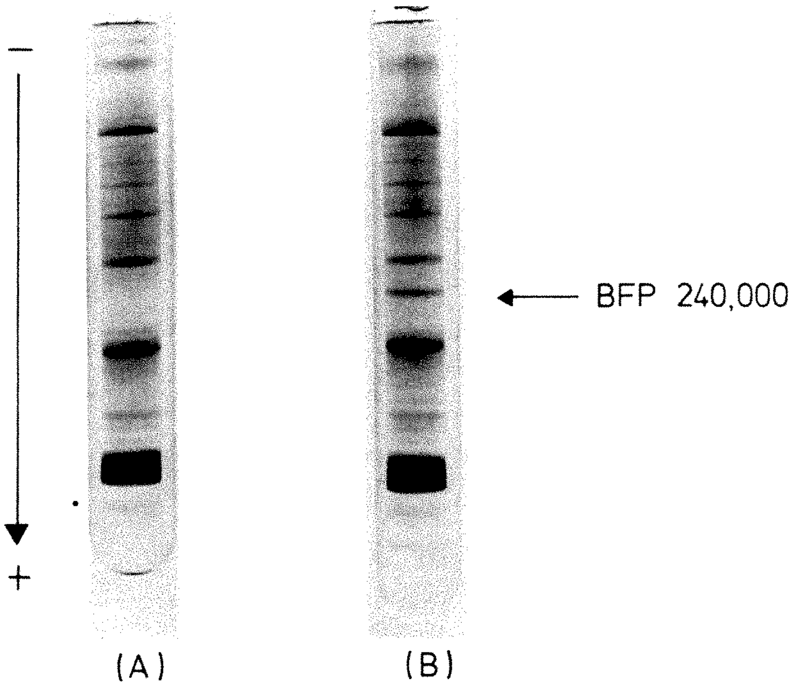

FIGURE 1. Polyacrylamide-gel gradient electrophoresis (linear gradient from 3·0% to 16·0% in Tris/HCl buffer system pH 8·4) (A) of the serum; (B) of the blister fluid.

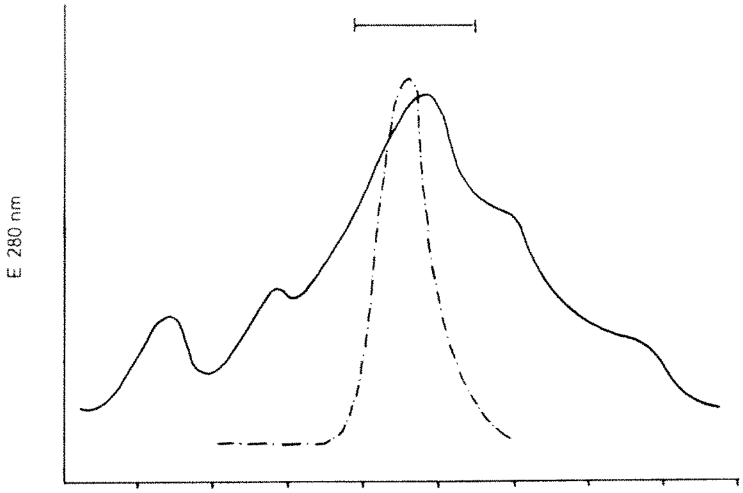

FIGURE 2. Elution profile of blister fluid on biogel A-0·5 m Equilibration with 0·02 mol/l potassium-phosphate buffer (pH 7·2). (——) protein (E 280 nm); (· – · – ·) BFP 240,000.

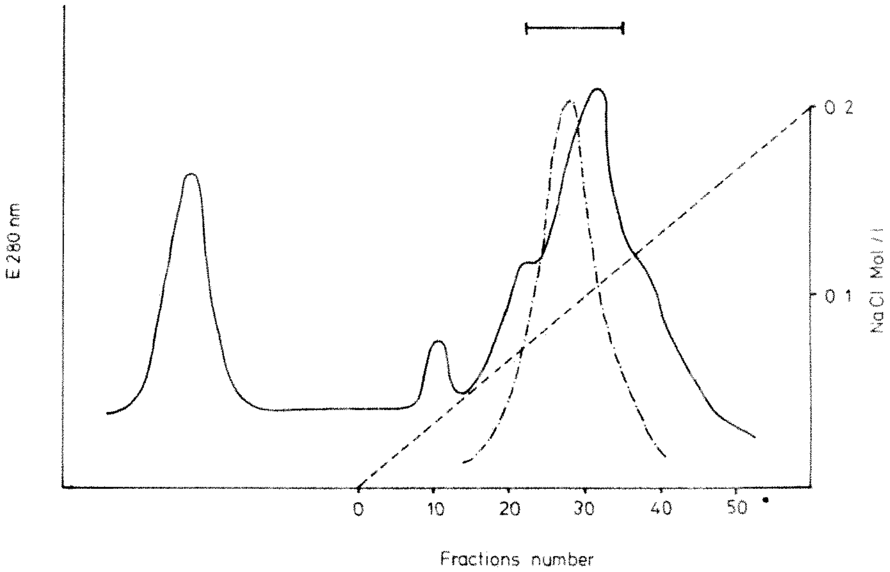

FIGURE 3. Elution profile of BFP 240,000 rich fractions 39–55 after biogel A-0·5 m chromatography (see Fig. 2) on DEAE-Sephadex A-50. Elution with a linear gradient of NaCl from 0–0·2 mol/l. (——) protein (E 280 nm); (· – · – ·) BFP 240,000.

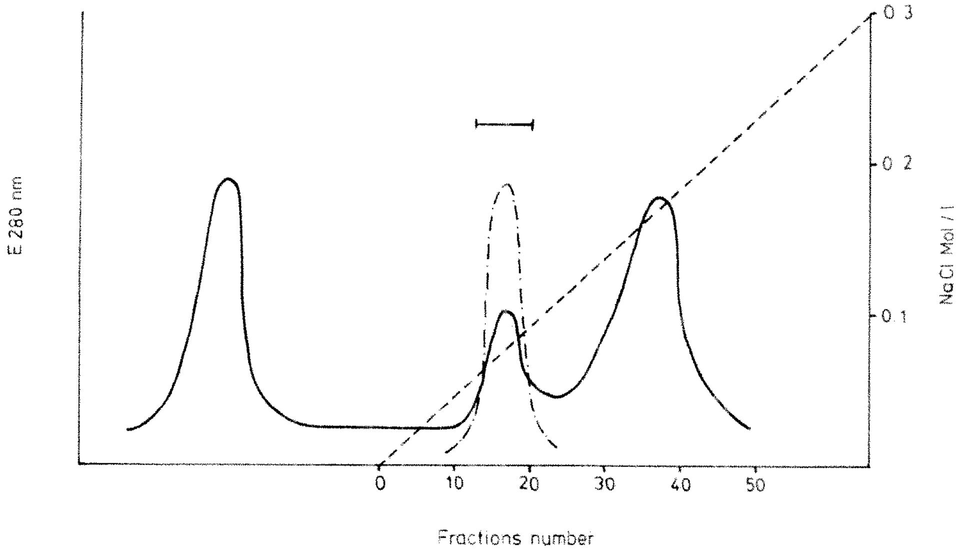

FIGURE 4. Elution profile of BFP 240,000 rich fractions 22–35 after ion exchange chromatography on DEAE-Sephadex A-50 (see Fig. 3) on CM-Cellulose. Elution with a linear gradient of NaCl from 0–0·3 mol/l. (——) protein (E 280 nm); (– · – · –) BFP 240,000.

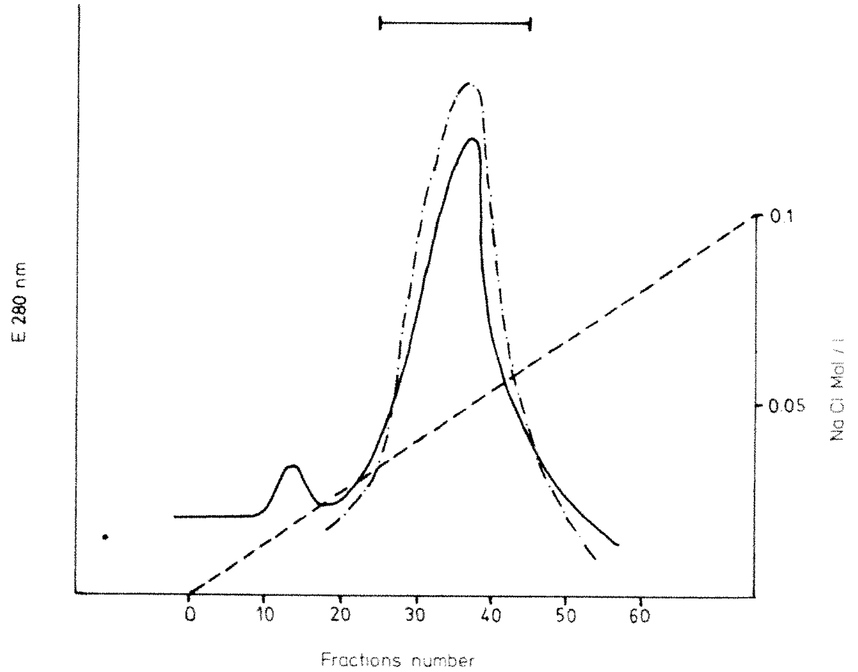

FIGURE 5. Elution profile of BFP 240,000 rich fractions 12–20 after ion exchange chromatography on CM-Cellulose (see Fig. 4) on DEAE-Sephadex A-50. Elution with a linear gradient of NaCl from 0–0·1 mol/l. (——) protein (E 280 nm); (– · – · –) BFP 240,000.

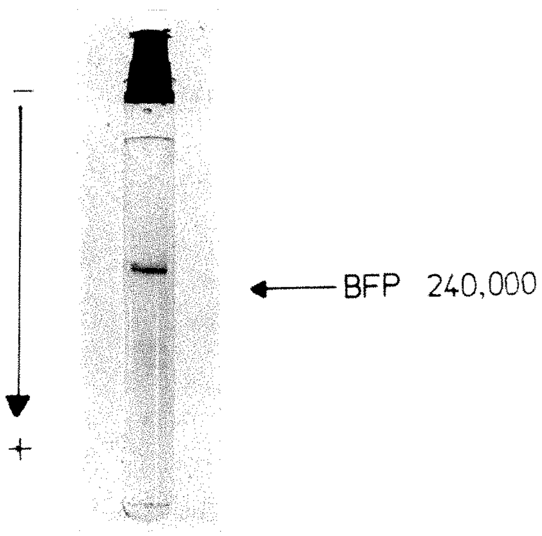

Fig. 6.

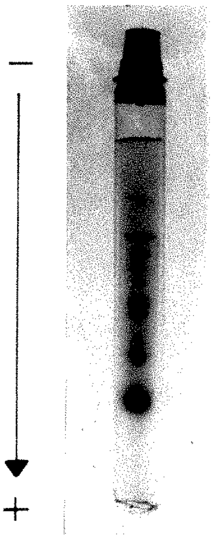

Fig. 7.

FIGURE 6. Polyacrylamide-gel gradient electrophoresis (linear gradient from 3·0% to 16·0% in Tris/HCl buffer system pH 8·4) of BFP 240,000 after rechromatography on DEAE-Sephadex A-50 and $(NH_4)_2SO_4$ precipitation of BFP 240,000 rich fractions 22–44 (see Fig. 5).

FIGURE 7. SDS-polyacrylamide-gel electrophoresis (0·1% SDS, 7·5% gel, 0·1 mol/l Na-phosphate buffer pH 7·0, 8 mA/tube of BFP 240,000.

### *Protein distribution pattern of serum and blister fluid*

As shown in Fig. 1 the protein distribution pattern of the serum and blister fluid, determined by PAA-gel gradient electrophoresis, was nearly identical except for one more concentrated fraction (BFP 240,000) in the blister fluid.

### *Preparation of blister fluid protein mol. wt 200,000 (BFP 240,000)*

*Step* 1: 5 ml blister fluid were applied to a column (K16/100) of biogel A-0·5 m, equilibrated with 0·02 mol/l potassium phosphate buffer (pH 7·2) at a flow rate of 10 ml/h (elution profile see Fig. 2).

TABLE 1. Precipitation of BFP 240,000 by anti-human-sera and BMZ-Ab sera from patients with bullous pemphigoid in the Ouchterlony technique (see Methods)

| Anti-human-sera | Precipitation |
|---|---|
| Polyvalent | + |
| $\gamma$-Globulin | + |
| C3/C3c | + |
| BMZ-Ab sera (titre 1:160) | + |
| IgG, IgM, IgA, IgD, IgE | − |
| Albumin | − |
| Transferrin | − |
| Fibrinogen | − |
| Haptoglobin | − |
| $\beta$-Lipoprotein | − |
| c-Reactive protein | − |
| Ceruloplasmin | − |
| $a_1$-Antitrypsin | − |
| Factor B | − |
| C1-esterase inhibitor | − |
| C1q-component | − |
| Normal sera | − |

*Step* 2: The BFP 240,000 rich fractions (fraction 39–55) from the gel chromatography step 1 were pooled and dialysed against 0·02 mol/l Tris/HCl buffer (pH 8·6) and subjected to ion exchange chromatography on a column of DEAE-Sephadex A-50 equilibrated with the same buffer. Elution was performed with a linear gradient of NaCl from 0–0·2 mol/l (elution profile see Fig. 3).

*Step* 3: The BFP 240,000 rich fractions (fraction 22–35) were pooled and dialysed against 0·02 mol/l potassium phosphate buffer (pH 6·0) and bound to CM-Cellulose. The bound material was eluted with a linear gradient of NaCl from 0–0·3 mol/l (elution profile see Fig. 4).

*Step* 4: The BFP 240,000 rich fractions (fraction 12–20) were pooled and dialysed against 0·02 mol/l Tris/HCl buffer (pH 8·2) and once more subjected to ion exchange chromatography on DEAE-Sephadex A-50. Elution was performed with a linear gradient of NaCl from 0–0·1 mol/l (elution profile see Fig. 5).

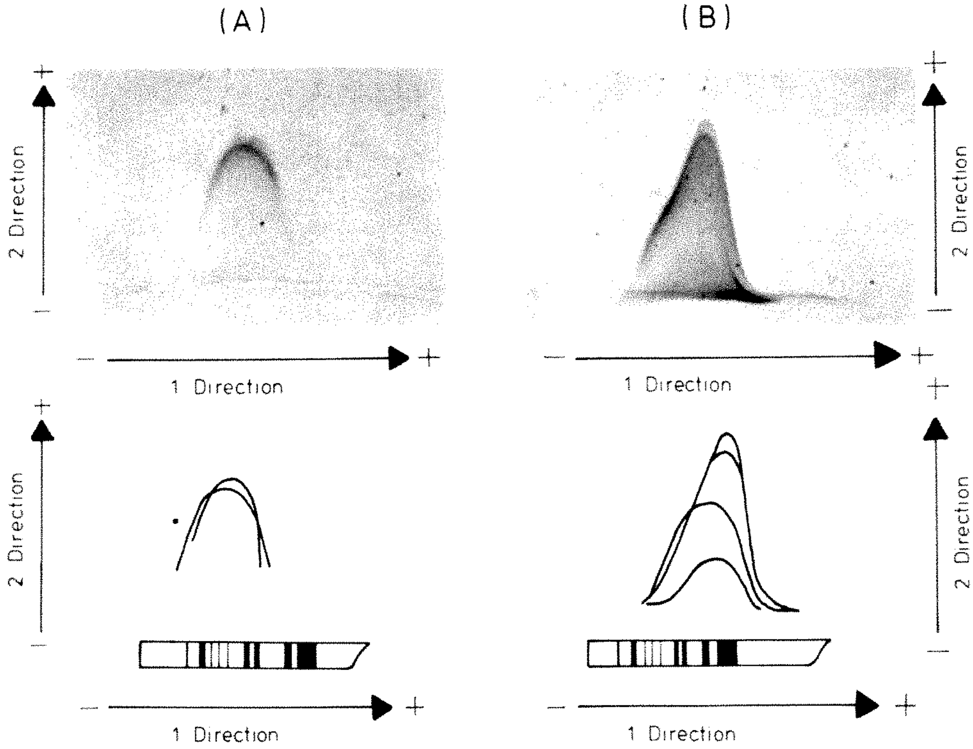

FIGURE 8. Modified two-dimensional Laurell immunoelectrophoresis of BFP 240,000. 1. Direction: SDS-electrophoresis (0·1% SDS, 7·5% PAA). 2. Direction: electroimmunodiffusion in agarose gel containing. (A) anti-C3/C3c serum; (B) BMZ-Ab serum from patients with bullous pemphigoid. For experimental conditions see Materials and Methods.

*Step* 5: The BFP 240,000 rich fractions (fraction 24–44) were pooled and the proteins were precipitated by means of 80% $(NH_4)_2$ $SO_4$. The precipitate was dialysed against 0·1 mol/l Tris/HCl buffer (pH 7·2) and then a PAA-gel gradient electrophoresis was carried out. The protein (BFP 240,000) showed only one homogenous band (Fig. 6).

## *Properties of the BFP 240,000*

*Molecular weight determination.* The molecular weight of pure protein (BFP 240,000) was about 240,000 in comparison with BSA-monomer, BSA-dimer, catalase-monomer and catalase-dimer as reference proteins determined by means of analytical PAA-gel gradient electrophoresis.

*Immunological and physicochemical characterization.* (1) As shown in Table 1 the BFP 240,000 is precipitated by means of the Ouchterlony technique by the following anti-human sera: polyvalent, γ-globulin, C3/C3c and sera from patients with bullous pemphigoid containing BMZ-Ab. (2) If the BFP 240,000 is submitted to SDS-electrophoresis, the protein which is homogeneous in the PAA-gel gradient electrophoresis (Fig. 6) shows a heterogeneous protein distribution pattern (Fig. 7). (3) To estimate how many SDS protein subunits are precipitated by anti-C3/C3c serum and BMZ-Ab serum, respectively, the modified Laurell technique was carried out. As shown in Fig. 8 two protein subunits were precipitated by anti-C3/C3c serum and two other protein subunits by BMZ-Ab serum.

## DISCUSSION

The nearly identical protein distribution pattern of the blister fluid compared with serum of the same patients with bullous pemphigoid, determined by the PAA-gel gradient electrophoresis, may be the result of an increased permeability of the blood vessels as a consequence of the activation of the classical and alternative complement pathways (Provost & Tomasi, 1973; Jordon *et al.*, 1975). However, a more concentrated protein fraction (BFP 240,000) in the blister fluid is remarkable as shown in the PAA-gel gradient electrophoresis (Fig. 1). After its isolation this protein exhibited a molecular weight of about 240,000. By further investigations we could demonstrate that this protein, which appeared homogeneous in its native form (Fig. 6), could be split into a heterogeneous spectrum of subunits by denaturing conditions (Fig. 7). Two of these subunits are probably C3 fragments, since they form specific immunoprecipitates with anti-C3/C3c sera (Table 1, Fig. 8A). Probably two other subunits are BMZ-Ag, since they form specific immunoprecipitates with sera from patients with bullous pemphigoid containing BMZ-Ab (Table 1, Fig. 8B). The nature of further subunits is still unclear, however, especially whether they are non-antigenic BMZ-split-products or probably other complement split-products.

The occurrence in blister fluid of free BMZ-Ag and BMZ-Ab which are not bound to immune complexes might be explained by an inequality in the concentrations of BMZ-Ag and BMZ-Ab. Jordon & McDuffie (1976) could find immune complexes in the blister fluid of patients with bullous pemphigoid. It is unlikely, however, that the BFP 240,000 represents immune complexes, because it did not form immunoprecipitates with anti-Ig sera (Table 1).

On the basis of the increased permeability of the blood vessels the BFP 240,000 containing BMZ-Ag may penetrate into blood circulation and form BMZ-Ag-BMZ-Ab complexes with BMZ-Ab. Their existence in serum has been proved by cleavage experiments in a previous study (Herrmann *et al.*, 1978).

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# B and T lymphocytes in lymphoproliferative diseases of the skin

## EFFECT OF A THYMIC HORMONE ON THE IMMUNOCOMPETENCE OF T LYMPHOCYTES

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## SUMMARY

Cell-mediated immunity using the E-rosette technique, the graft-versus-host reaction and intradermal skin tests were investigated in two groups of patients, twenty-two with benign lymphoproliferative diseases of the skin (lymphocytic infiltration of the skin and lymphadenosis benigna cutis) and nine with malignant lymphoproliferative diseases (mycosis fungoides and lymphoma cutis). The effect of thymic hormone upon T lymphocytes which proved to have impaired functional capacity was investigated. Seventeen of the twenty-two patients with benign lymphoproliferative diseases were found to have intact cellular immunity whereas seven of the nine patients with malignant disease proved to be immunodeficient. The determination of immune competence may be of help in establishing the correct diagnosis in doubtful cases and might also be useful in the follow-up of these patients. The improvement in functional capacity of T lymphocytes resulting from their interaction with a thymic hormone indicates that it holds therapeutic promise.

Studies carried out on the function of the cell-mediated immune system in patients with malignant lymphoproliferative diseases of the skin such as mycosis fungoides (MF) (Lutzner *et al.*, 1975), Sézary syndrome (Nordqvist & Kinney, 1976) and non-Hodgkin lymphoma (Aisenberg, 1973) have indicated at least a partial immunodeficiency in most cases. To the best of our knowledge, however, no reports on the immunocompetence of patients with benign lymphocytic diseases of the skin, as lymphocytic infiltration of the skin (LIS) and lymphadenosis benigna cutis (LABC) have been published.

Our study was carried out to determine the quality of the cell-mediated immune response in these benign lymphoproliferative diseases as compared with that in the malignant diseases mycosis fungoides

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and lymphoma cutis (LC). Also, the effect of thymic humoral factor (THF), a thymic hormone, on the function of T cells from those patients who proved to be immunodeficient was also investigated. The THF isolated by Trainin from calf thymus has been found to restore immunoreactivity in animals thymectomized at birth (Trainin *et al.*, 1975).

## MATERIALS AND METHODS

The patients used in this study were seventeen (six women, eleven men; age range 35–71 years) with lymphocytic infiltration of the skin; five (three women, two men; 29–73 years) with lymphadenosis benigna cutis; four (four men; 39–75 years) with lymphoma cutis; and five (two women, three men; 36–73 years) with mycosis fungoides. The diagnosis was determined by clinical and histopathological criteria.

Fourteen of the patients with LIS and three of those with LABC had the typical skin lesions at the time of the investigation whereas the others were in remission. Of the four patients with LC, two had a poorly differentiated lymphocytic lymphoma and two a lymphocytic-histiocytic lymphoma; at the time of investigation three showed clinical lesions whereas the fourth was asymptomatic due to radiation therapy. Among the five patients with MF, four were in stage I of the disease and one patient was in stage II.

Each patient had a total blood count, tests of hepatic and renal function, and urinalysis.

Samples of peripheral blood were obtained from the patients and also from thirty-two healthy volunteers from the medical staff of the hospital who served as a control group. The lymphocytes were isolated by the Ficoll-Hypaque sedimentation technique. The number of T lymphocytes was determined using the spontaneous rosette formation test (Jondal, Holm & Wizgell, 1972) and the number of B lymphocytes by the EAC-rosette test (Bianco, Patrick & Nussenzweig, 1970). The function of the T cells was assessed by means of the graft-versus-host reaction (GVHR) (Shohat & Joshua, 1976). A reaction measuring 3–5 mm in diameter was considered as positive, 1–2 mm as weak and a reaction of 0·5 mm as negative. In those cases in which immunocompetence was found to be impaired, the effect of THF was determined on the basis of the GVHR obtained after a dose of 100 gamma THF had been injected on 3 consecutive days into rats in which the lymphocytes of the immunodeficient patient had been injected intradermally (Shohat *et al.*, 1978).

Intradermal skin tests were performed in all patients using four recall antigens, Candidin (1:50), Trichophytin (1:50), Tuberculin (1:1000) and Staph. toxoid (1:50), a reaction being considered positive when there were erythema and induration of 5 mm in diameter 72 h after the injection.

## RESULTS

Routine blood tests and urinalysis were normal in all of the patients.

### *B and T lymphocytes in peripheral blood*

Table 1 compares the mean values obtained in the four groups of patients with those of the controls. The mean percentage of T lymphocytes was significantly lower than that of the controls in the patients with LIS ($P < 0{\cdot}01$), LC($P < 0{\cdot}02$) and MF ($P < 0{\cdot}05$), whereas the mean absolute number of T cells was significantly lower only in the patients with LC ($P < 0{\cdot}05$) and in patients with MF ($P < 0{\cdot}005$). The mean percentage of B lymphocytes was significantly lower than that of the controls in the patients with LC ($P = 0{\cdot}05$) and in patients with MF ($P < 0{\cdot}05$). There was no significant difference between the absolute number of B cells in the various groups of patients and those in the controls, with the exception of the LC group, for which the statistical difference was borderline ($P = 0{\cdot}06$).

TABLE 1. Means ± standard deviations of T and B lymphocytes in peripheral blood of patients

| Disease | No. of patients | Percentage of T lymphocytes | Total number of T lymphocytes mm$^{-3}$ | Percentage of B lymphocytes | Total number of B lymphocytes mm$^{-3}$ |
|---|---|---|---|---|---|
| Lymphocytic infiltration of the skin | 17 | 49·4 ± 9·72 | 1140·8 ± 302·0 | 15·2 ± 6·62 | 380·7 ± 253·3 |
| Lymphadenosis benigna cutis | 5 | 54·2 ± 5·95 | 918·8 ± 129·6 | 17·6 ± 6·77 | 292 ± 102·8 |
| Lymphoma cutis | 4 | 44·8 ± 12·56 | 744·8 ± 200·6 | 13·3 ± 7·59 | 199 ± 39·2 |
| Mycosis fungoides | 5 | 49·4 ± 8·53 | 966·8 ± 535·11 | 15·4 ± 6·19 | 328·2 ± 222·2 |
| Control | 32 | 58·3 ± 8·96 | 1262·6 ± 461·5 | 21·5 ± 3·35 | 400 ± 150·0 |

### *Graft-versus-host reaction*

Among the controls, thirty had a positive GVHR and only two a negative GVHR. Of the twenty-two patients with LIS and LABC only four had a negative GVHR, whereas of the nine patients with LC and MF; seven had a negative GVHR. In ten of the eleven patients (three with LIS, four with MF and three with LC) whose lymphocytes had failed to elicit a positive GVHR in rats, additional samples of the patient's immunodeficient lymphocytes were injected into rats, following which THF was administered. In the lymphocytes obtained from six of the patients an improvement in immunocompetence was seen to result, i.e. a conversion from a negative or weak reaction into a positive GVHR was observed.

### *Intradermal skin tests*

Among the seventeen patients with LIS only three proved to be totally anergic and these also had a negative GVHR. Of the five patients with LABC, two were anergic. Three of the four patients with LC and four of the five with MF were anergic. Thus five of the twenty-two patients with benign disease and seven of the nine with malignant disease were anergic.

## DISCUSSION

We observed the difference in immunocompetence in patients with benign lymphoproliferative diseases of the skin and those with malignant lymphoproliferative diseases of the skin. Most of the patients with LIS or LABC had normal immunoreactivity. There was a partial correlation between clinical stage and immunocompetence, since among the five patients who were in remission, one had a negative GVHR and another two proved to be anergic. In contrast all of the patients with MF or LC presented some evidence of immunodeficiency: in more than two-thirds there was a negative GVHR and complete anergy to the intradermal skin tests, demonstrating severe impairment of delayed hypersensitivity. Of particular interest are the results obtained in patients with LC. The immunodeficiency in non-Hodgkin lymphomas described by other investigators has been found primarily to involve humoral immune function (Aisenberg, 1973). Depressed delayed hypersensitivity has also been reported but usually in relation to a general debility (Miller, 1971). In contrast, we found complete anergy in three of these patients before they received treatment and while they were still in a satisfactory general condition.

Difficulty is sometimes encountered in interpreting the histological picture in cases of LIS and LABC. The lymphocytic infiltrate in LABC may resemble nodular lymphoma or mixed histiocytic-lymphocytic lymphoma and the findings in LIS may not be distinguishable from those in well-differentiated lymphocytic lymphoma (Lever & Schaumburg-Lever, 1975). Our results suggest that in doubtful cases investigation of the immunological system may aid in reaching the correct diagnosis.

A small number of patients with LIS and LABC develop malignancy in late stages of the disease (Bafverstedt, 1968; Lever & Schaumburg-Lever, 1975). Whereas the initial diagnosis in these cases may have been incorrect, it is also possible that there was a malignant transformation of the previously benign process. The results of our study suggest that cases in which the histopathological diagnosis is benign but immunocompetence is impaired should be followed up to detect the development of actual malignancy.

THF is a hormone which induces maturation of T cells and thus improves immunoreactivity. It was used recently in the treatment of a 3½-year-old boy seriously ill with adenovirus influenza who was shown to have a severe immunocellular deficiency, and resulted in a dramatic clinical improvement and restoration of cellular immune competence (Varsano *et al.*, 1977). Therapeutic trials have also been carried out in cases of subacute sclerosing panencephalitis (Varsano *et al.*, 1976) and in children with acute lymphocytic leukaemia and varicella (Zaizov *et al.*, 1977).

In our work THF was found to improve the function of T lymphocytes in several of the patients tested, findings which confirmed results obtained in previous studies by Shohat *et al.* (1978).

Whether the administration of THF may lead to an improved prognosis in patients with lymphoproliferative diseases of the skin remains to be seen in future investigations.

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# A simple method for correlating observations on skin at the light and electron microscopic levels

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## SUMMARY

A method is described which allows the embedding of 6 mm cutaneous punch biopsies for subsequent light and electron microscopic examination. Sections of 20 mm$^2$ cut at 0·5–1 $\mu$m are stained for light microscopy. The subtle tinctorial affinities, which approach those achieved on haematological smears, and the preservation of cellular detail frequently make subsequent ultrastructural examination unnecessary. However, when required, a special technique for trimming and resectioning the tissue blocks allows a good correlation between light and electron microscopic observations.

Important advances in the understanding of skin diseases have recently been made by new histological techniques (Askenase, 1977; Dvorak *et al.*, 1977). These techniques require the use of 0·5–2 $\mu$m histological sections of large surface area (9 mm$^2$ (Barr *et al.*, 1978) to 40 mm$^2$ (Dvorak *et al.*, 1977)) which may be obtained from skin biopsies embedded in epoxy resin. In this way, improvement of histological and cytological analysis is possible, but it has been difficult (Dvorak *et al.*, 1977) or impossible (Barr *et al.*, 1978) to achieve an exact correlation between results obtained by light and electron microscopy.

We describe here a new method which permits the cutting of 0·5–1 $\mu$m sections of large surface area (20 mm$^2$) for light microscopy and the subsequent use of the same block to obtain ultra-thin sections of an area of special interest. In this way, it is possible to correlate light and electron microscopic observations.

Three staining techniques are proposed for 0·5–1 $\mu$m epoxy resin sections which give a precise picture of the basal membranes and a very delicate appreciation of the cellular acidophilia and basophilia. The quality of cytological details obtained thus under the light microscope tends to make ultrastructural observation unnecessary. When electron microscopy is desired the precise correlation with light microscopy may greatly enhance its value.

## MATERIALS AND METHODS

### *Biopsies*

Punch biopsies (6 mm) were obtained after local anaesthesia with 2% xylocaine without adrenalin.

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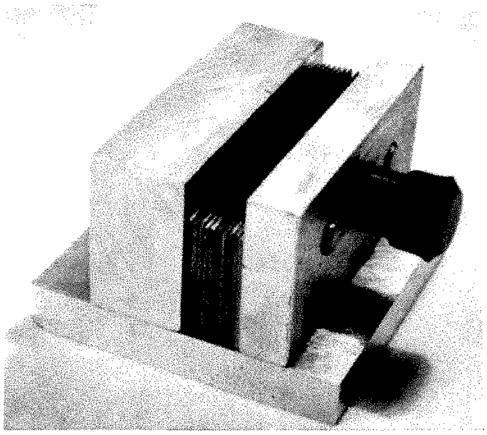


FIGURE 1. A newly designed hand microtome. Razor blades are separated by steel plates of 0·6 mm thickness.

### Fixation

The biopsy was placed in 10 ml of Karnovsky's fixative, (prepared less than 8 days previously), and diluted by half in 0·1 mol/l cacodylate buffer at pH 7·4 just before use (Karnovsky, 1967). After 15 min of fixation, the biopsy was cut into slices of 0·7 mm thickness, perpendicular to the cutaneous surface, with a newly designed hand microtome (Fig. 1). This microtome was constructed with ten stainless steel razor blades. The blades were separated from each other by stainless steel spacers, each of 0·6 mm thickness. These spacers were of the same length as the razor blades (43 mm) but slightly narrower in width (19 mm). In this way it was possible to put the biopsy, epidermis below, on the cutting edge of the blades and to press down with a piece of elderwood. The slices so cut were then taken off with a narrow spatula. The total duration of the fixation was 5 h at 4°C. The fragments were then washed twice for 15 min and subsequently for 12 h at 4°C in cacodylate buffer (0·1 mol/l, pH 7·4). An optional post-fixation with $OsO_4$ for 2 h at room temperature permitted, if desired, a better preservation of membranes for ultrastructural investigations. This post-fixation was carried out in $OsO_4$ (1·33% in the cacodylate buffer 0·1 mol/l, pH 7·4) and followed by two washes of 15 min each in cacodylate buffer (0·1 mol/l, pH 7·4).

### Inclusion

The resin used was that described by Bryon (1976) slightly modified for our purposes. The epon mixture contained: V.C.D. (vinyl cyclohexene dioxide LADD) 11 g; EPON 812 (LADD) 50 g; N.S.A. (nonenyl succinic anhydride LADD) 67 g. This mixture could be stored at 4°C for several weeks before use. DMP 30 (Tri (dimethylaminomethyl) phenol LADD) was added just before use at the rate of 0·18 g per 10 ml of mixture. The dehydration of the fragments in ethanol (70%, 15 min; 95%, 15 min; 100%, three times 15 min each) was followed by three washes in propylene oxide (three times for 15 min each). Thereafter, the fragments were transferred for 1 h into a mixture (1 vol/1 vol) of resin/propylene oxide and for 12 h in a mixture (2 vol/1 vol) resin/propylene oxide. Finally, the fragments were placed in pure resin for 4 h under vacuum.

### Orientation and polymerization

The biopsy fragments were then placed flat on a glass slide. Gelatin capsules of 0·4 ml were filled with resin and turned upside down on the fragments which were then set perpendicularly towards the gelatin capsules. After 12 h of polymerization at 37°C and 12 h at 60°C the gelatin capsules were taken off by quickly heating the slide with a small flame; the capsules were then incubated for 24 h at 60°C.

### *Sectioning*

*Light microscopy.* Sections (0·5–1 μm) of large surface area (20 mm²) were made with an ultramicrotome LKB IV and glass knives of 5 mm width. A good reproducibility in thickness of the sections was obtained with an inclination of the knife of 1° and a declivity speed of the block of 0·5 mm/s.

The sections were collected on water with a ring and transferred into a bath of demineralized water at 60°C. The sections were then placed on clean glass slides and dried for 3–12 h at 60°C.

*Electron microscopy.* The ultramicrotome LKB IV has an original optical system which made it possible to localize precisely the area for ultrastructural analysis on the block containing the sections of large surface.

A Mesa of 20 μm height was then carved (Reid, 1975), and the first ultra-thin sections which were cut permitted us to look at the ultrastructure of the cell or the group of cells which were seen under light microscopy on the last semi-thin section of large surface. The sections were collected on grids of 75 mesh overlayed with Formvar and carbon.

### *Staining*

*Light microscopy.* The best staining was always obtained after dissolving the resin in a freshly prepared solution of: saturated sodium methanol alcoholate, methanol and acetone in the proportions 1:1:1 by volume. This was followed by three washes in 1:1 (vol/vol) acetone and methanol and by three in 1:1 (vol/vol) acetone/water. The sections of 1 μm were left for 1 min in each wash. The slides were then placed in demineralized water for 5 min. Subsequently various staining techniques were undertaken. The following three were used routinely:

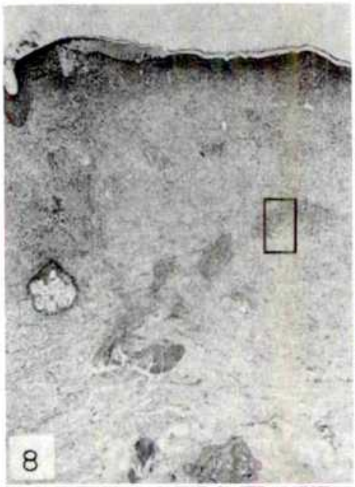
(1) Alcoholic eosin–slow Giemsa: A 0·2% solution of eosin Y (RAL Pointet Girard) in 60% ethanol was applied for 2 min on the tissue sections. The excess solution was then removed and the slide placed, sections below, in a Laveran's box containing a 5% solution of slow Giemsa (RAL) freshly prepared in phosphate buffer at pH 6·8. This Giemsa staining was performed twice consecutively, 20 min each time. The sections were then rinsed in phosphate buffer (pH 6·8) and dehydrated in butanol (15 s) and N-butylic alcohol (15 s). This was followed by two changes in xylene (1 min each), and the samples were then mounted in Eukitt (Kindler).

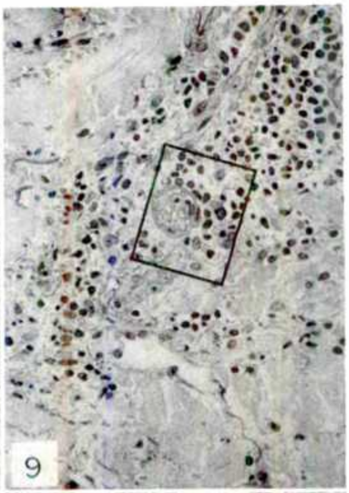
(2) May-Grunwald Giemsa: A 25% solution of May-Grunwald (RAL), freshly prepared in phosphate buffer at pH 6·8, was applied to the tissue sections for 15 min. After this, the samples were processed as above.

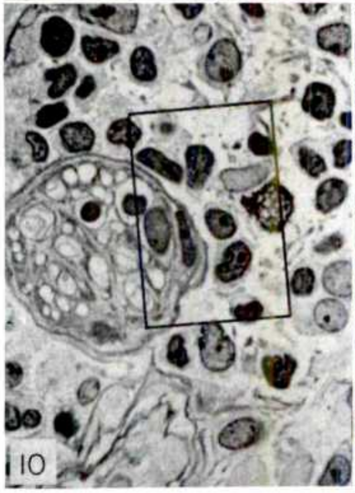
(3) Giemsa in acid solution (Tulliez, personal communication): The samples were placed vertically for 3 h in a glass jar containing a 5% solution of slow Giemsa (RAL) freshly prepared in acetic acid ($10^{-6}$ mol/l). Rinsing was performed with acetified water and the dehydration and mounting as before.

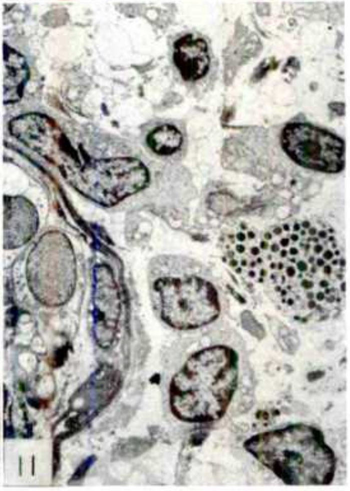
*Photography.* Photomicrographs were prepared with a Zeiss Photomicroscope equipped with Zeiss planapochromat objectives.

*Electron microscopy.* The staining of ultra thin sections was made on grids. These were placed, sections below, for 20 min in the dark on a drop of saturated solution of uranyl acetate (TAAB) in 70% ethanol. This solution was prepared and filtered in the dark just before use. The rinsing was done with









FIGURES 8–10. A 1 μm section of large surface area seen at × 20 (Fig. 8); × 200 (Fig. 9); × 800 (Fig. 10).

FIGURE 11. Ultrastructure of the first ultra-thin section next to the 1 μm section of large surface area (× 2600). Note the close correlation with the light micro-photography. The shapes of nuclei are slightly modified. For example the nucleus of the cell situated above the mastocyte is disappearing.

bidistilled water. The grids were then turned upside down and left for 2 min on a drop of lead citrate solution according to Reynolds (1963).

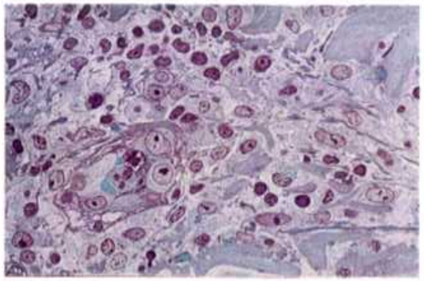
## RESULTS

The 1 μm sections of large surface which had been fixed and stained (see Figs 2–7) allowed preservation and clear visualization of the structures of the epidermis and dermis. As seen below, the precision of the details often made ultrastructural examination unnecessary.

Each of the staining techniques has specific advantages:

The alcoholic eosin-slow Giemsa (Figs 2 and 3) precisely reveals the basophilia and acidophilia of cellular structures, and is particularly useful for plasma cells and eosinophil granules. Basal membranes

(2)



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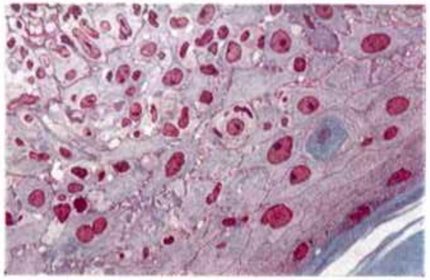

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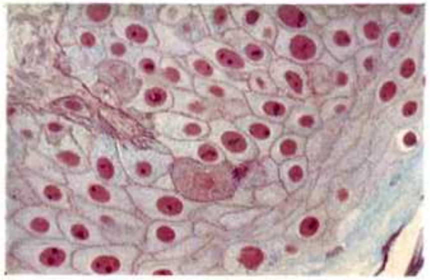

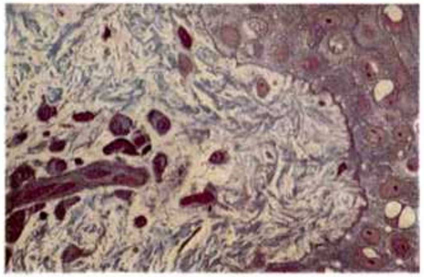

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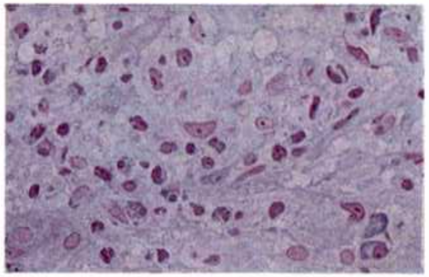
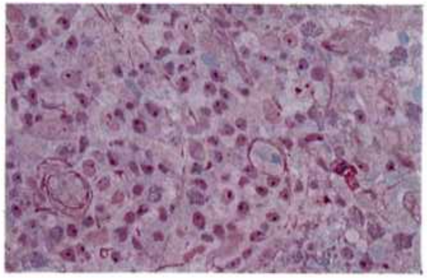


(6)



(7)

FIGURE 2. Alcoholic eosin-slow Giemsa (× 375). Dermal perivascular infiltrate. Note the basal membrane of capillaries, the purple basophil on the left and the numerous eosinophils above and below it. FIGURE 3. Alcoholic eosin–slow Giemsa (× 375). Chronic and polymorphic dermal infiltrate (mycosis fungoides). Note the numerous Lutzner cells. At the top numerous eosinophil leukocytes and two plasma cells on the left. The collagen is slightly basophilic. FIGURE 4. May-Grunwald Giemsa (× 375). The staining is less subtle than in Figs 2 and 3 but with more contrast. Two plasma cells are clearly seen under the capillary. FIGURE 5. Giemsa in acetified water (× 375). Two mitoses and a cell with a cerebriform nucleus in the epidermis. Note the strong staining of the basement membrane of the epidermis and of the intercellular substance joining keratinocytes. FIGURE 6. Giemsa in acetified water after post fixation with $OsO_4$ (× 375). A strong epidermotropism. Note the precision of cellular details, the strong staining of keratin, keratohyalin and of the intercellular substance joining keratinocytes. Down on the right the basement membrane of the epidermis. FIGURE 7. Giemsa in acetified water after post fixation with $OsO_4$ and 1% periodic acid for 30 min (× 375). A perivascular infiltrate. Note the purple basophil leukocyte and the trinucleated polymorphnuclear in the vessel, the binuclear eosinophil leukocyte on the left.

are very well stained. These features make this stain very useful for analysing dermal cellular infiltrates. Epidermal staining is sometimes a little weak. Erythrocytes become basophils after their short passage in sodium methanol alcoholate.

May-Grunwald Giemsa (Fig. 4) gave good staining of epidermis, whereas basal membranes were less clear. The staining of dermal infiltrates was slightly less subtle but cytoplasm was sometimes seen with more contrast. Thus, this appears to be a good technique for observing whole structures of the skin.

Giemsa in acetified water (Fig. 5) gave very clear staining of the epidermis with a particularly intense red staining of the inter-cellular material between keratinocytes. Basal membranes were also very well distinguished. The staining of collagen was intense, but that of dermal infiltrates less so.

Post-fixation with $OsO_4$ altered the tinctorial affinities, especially the acidophilia and basophilia. This did not prevent polychromatic staining required for precise cytological analysis. After post-fixation with $OsO_4$, staining with acid Giemsa produced excellent results (Figs 6 and 7). The best results were obtained on sections of 0·5 μm. When the blue colour was too intense, treating in 1% periodic acid for 5–30 min before staining greatly improved the results.

The transition from optical to ultrastructural observation was generally easy, not only with tissue treated in Karnovsky's fixative alone, but also with that post-fixed with $OsO_4$ (Figs 8–11) In blocks fixed only with Karnovsky's fixative, membranes were seen only negatively in ultrastructure, and it was sometimes difficult to study dense cellular infiltrates and intra-cellular organelles such as Langerhans granules.

The resin gave excellent results for cytological studies at light and electron microscopic levels. For special purposes (for example histo-enzymological studies at the ultrastructural level) it may be convenient to increase the hardness of the resin. This may be done by increasing the V.C.D. and decreasing the EPON 812 in the mixture. Thus the cutting of 1 μm sections of large surface area remained satisfactory.

## DISCUSSION

Some authors have used post-fixation with $OsO_4$ and did not dissolve the resin (Dvorak, 1977). The morphological results were good but very different from haematological smears. Above all, this type of staining has been used for analysing structures of strong tinctorial affinity, such as nuclei, granules of polymorphonuclear leukocytes and mastocytes.

With our techniques we obtained clear topographical and cytological visualization and staining as near as possible to that achieved with May-Grunwald Giemsa on haematological smears.

Skin is a polymorphic tissue, and at the ultrastructural level it is not possible, even with 1 μm sections of small surface area, to obtain sufficiently exact analysis of the diversity and topography of lesions. In addition, the choice of the region to be examined is very limited.

Sections of large surface area give more possibilities for detailed analysis. Unfortunately, it has been difficult (Dvorak *et al.*, 1977; Grimley, Albrecht & Michelitch, 1965) or impossible (Barr *et al.*, 1978) until now to follow up the observations at the ultrastructural level of one special area.

Resins employed by other authors had been sufficiently soft to permit large sections but were not hard enough to allow ultra-thin ones (Dvorak *et al.*, 1977). Re-embedding of sections of large surface area allowed ultrastructural study (Grimley *et al.*, 1965); however, this technique is long and fastidious. The fixatives used in other studies were not compatible with ultrastructural examination (Barr *et al.*, 1978). Finally, the usual ultramicrotomes were not efficient enough to permit the cutting of sections of large surface area and of very precise thickness. Such precision was needed for reproducible staining.

Using skin fragments of large surface area but of 0·7 mm thickness permitted fixation that was compatible with ultrastructural observation. The very liquid resin used was well suited for embedding. Once polymerized, this resin was easily cut in large 1 $\mu$m sections and at the same time it was sufficiently hard to allow fine sections of good quality to be cut for electron microscopy. The ultramicrotome LKB IV had sufficient power to cut 0·2–2·5 $\mu$m sections of large surface area (20 mm$^2$). Its optical system permitted us to mark easily the area where a mesa should be cut. Thus, every area of interest at the light microscopic level could subsequently be studied ultrastructurally. Around the mesa, only 20–50 $\mu$m thickness of tissue block was used up. Thus it was possible to repeat several examinations on the same block by both light and electron microscopy.

Finally, it may be said that the quality of the results obtained by light microscopy with the fixatives and stains used here frequently made subsequent ultrastructural examination unnecessary. The precise correlation of the areas examined by light microscopy with those examined by electron microscopy greatly increased the interest of ultrastructural observation when it remained necessary. This technique can be applied on every polymorphic tissue (skin, kidney, lung, intestine) where correlation between light microscopic and ultrastructural observations may be extremely useful.

This technique is not very expensive in view of the quality and the quantity of information that can be collected on one single tissue sample. Various cytochemical, histo-enzymological and immunocytochemical reactions can be performed on samples embedded and cut as described here. Such techniques will be published later.

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# Benign and neoplastic eosinophilic staining cells: an immunofluorescence study

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## SUMMARY

Deposition of immunoglobulins, complement and fibrinogen on eosinophilic staining cells was investigated using direct immunofluorescence techniques. Serum factor deposition was detected on benign epidermal eosinophilic cells seen in pityriasis lichenoides et varioliformis acuta, sunburn erythema and, in addition, on subepidermal hyaline bodies in lichen planus; no such deposition occurred on neoplastic eosinophilic cells in Bowen's disease and squamous cell carcinoma. The qualitative findings of immunofluorescence microscopy seem to be different in inflammatory and malignant dermatoses.

Epidermal eosinophilic staining cells are known to be present in both benign and malignant dermatoses. The former conditions include pityriasis lichenoides et varioliformis acuta (Mucha-Habermann's disease) (Marks & Black, 1972), ultraviolet light-induced erythema (Daniels, Brophy & Lobitz, 1961), toxic epidermal necrolysis, fixed drug eruption (Komura, Yamada & Ofuji, 1969) and croton oil-induced epidermal injury (Takigawa, Komura & Ofuji, 1978). Neoplastic eosinophilic cells, often referred to as individual cell keratinization, are seen in Bowen's disease, squamous cell carcinoma and keratoacanthoma (Lever & Schaumburg-Lever, 1975). Histopathologically, both benign and neoplastic eosinophilic cells show a homogeneous and eosinophilic staining cytoplasm, occasionally associated with a condensed pyknotic nucleus. By means of electron microscopy, the structures of these two types of cells cannot be differentiated (Hashimoto, 1976).

In this study, we used fluorescence microscopy to investigate the occurrence of serum factor deposition on epidermal eosinophilic cells. Deposition of serum factors on subepidermal hyaline bodies in lichen planus was also studied, since hyaline bodies in lichen planus are considered to be of epidermal origin (Ueki, 1969). Immunopathological findings were compared between inflammatory and neoplastic conditions.

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## MATERIALS AND METHODS

### *Skin specimens*

Punch biopsies were obtained from four cases of Mucha-Habermann's disease, three cases of UV light-induced erythema, seven cases of lichen planus, four cases of Bowen's disease and three cases of squamous cell carcinoma. Skin specimens of UV-induced erythema were taken from human volunteers at 48 h after 6 min exposure to four tubes of fluorescent sunlamp (Toshiba FL 20SE) from a distance of 20 cm. The light source emits rays mainly between 280 and 320 nm (UV-B) peaking at 300 nm. Tissues were divided and half was immediately frozen in acetone–dry ice mixture and stored at −80°C until required. The other half was fixed in 10% formalin for routine haematoxylin and eosin (H & E) stain. Some of the sections were also stained with periodic acid-Schiff (PAS).

### *Direct immunofluorescence techniques*

Frozen tissues were sectioned at 5 μm on a cryostat and air dried. Unfixed sections were washed in three changes of 10 mM phosphate buffered saline (PBS) pH 7·4 for 30 min at 4°C. They were stained with FITC-labelled antisera for 30 min at 22°C. The conjugates used were rabbit anti-human IgG (total protein concentration (TPC) 4·4 mg/ml, F/P ratio 1·74 molar, Dakopatts laboratories), rabbit anti-human IgM (TPC 3·5 mg/ml, F/P ratio 1·94, Dakopatts), rabbit anti-human IgA (TPC 4·1 mg/ml, F/P ratio 1·40, Dakopatts), rabbit anti-human C3 (TPC 2·5 mg/ml, F/P ratio 1·54, Dakopatts), rabbit anti-human C1q (TPC 4·4 mg/ml, F/P ratio 2·12, Hoechst), and rabbit anti-human fibrinogen (TPC 4·1 mg/ml, F/P ratio 2·55, Dakopatts). All antisera were diluted 1:10 with PBS before use. After washing in PBS, the sections were mounted in buffered glycerol. All slides were observed with a Nikon epifluorescence microscope. After observation, sections were stained with H & E.

Specificity of the immunoreactions was confirmed as follows: sections were layered with (a) FITC-labelled swine anti-rabbit immunoglobulins (1:10 diluted with PBS, Dakopatts); (b) first unlabelled antisera to block specific antigenic sites and then stained with FITC-labelled antisera.

## RESULTS

### *Benign dermatoses*

Results of direct immunofluorescence techniques are summarized in Table 1. Purpuric papules obtained from two cases of Mucha-Habermann's disease showed eosinophilic cells mostly in the upper epidermis, including the parakeratotic horny layer. Significant deposition of IgM and fibrinogen was detected on eosinophilic cells (Fig. 1). Using light and fluorescence microscopy, these deposits could not be found on all of the eosinophilic cells shown in H & E restained frozen sections. Fluorescence

TABLE 1. Occurrence of the deposition of serum factors on eosinophilic staining cells

| Dermatoses | Total cases | No. of cases showing positive fluorescence for serum factors | | | | | |
|---|---|---|---|---|---|---|---|
| | | IgG | IgM | IgA | C3 | Clq | Fibrinogen |
| Pityriasis lichenoides et varioliformis acuta | 4 | 1 | 2 | 1 | 1 | 1 | 2 |
| UV light-induced erythema | 3 | 2 | 2 | 0 | 1 | 0 | 3 |
| Lichen planus | 7 | 4 | 6 | 2 | 3 | 1 | 7 |
| Bowen's disease | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Squamous cell carcinoma | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

for C1q and C3 was also shown in one of the cases and weak fluorescence for IgG and IgA was detected in the other case. Reactivity showed mostly a homogeneous and globular fluorescence. A ring shape pattern was occasionally seen, indicating that the deposition of serum factors may be localized in the cytoplasm but not in the nucleus. In addition, lamellar fluorescence for IgG and IgM and weak fluorescence for IgA, C1q and C3 were observed in parakeratotic horny layers. Papulonecrotic lesions of the other two cases showed only a few eosinophilic cells without detectable immunoprotein deposition. In all cases studied, no fluorescence was demonstrable at the basement membrane zone or around dermal vessel walls.

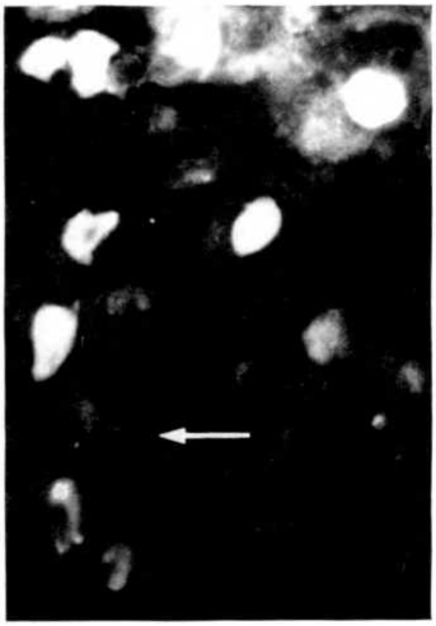


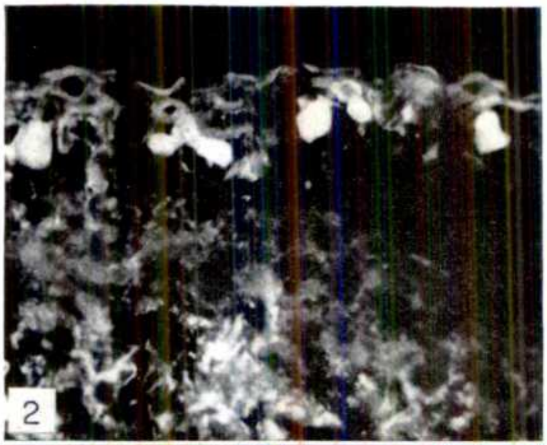
FIGURE 1. A frozen section of Mucha-Habermann's disease stained with FITC-labelled anti-human IgM. Homogeneous and globular fluorescence on epidermal eosinophilic cells was shown. Note an eosinophilic cell without any deposition (arrow) (× 680).

A number of sunburn cells were seen in epidermis of UV light-irradiated skin. Some of these cells were associated with serum deposits (Fig. 2). Fluorescence for fibrinogen was detected in all cases. Deposition of IgG and IgM was also seen in two cases, while C3 deposition was found in the other case.

In lichen planus, cytoid deposits of serum factors were localized on PAS-positive subepidermal hyaline bodies but not anywhere else (Fig. 3). There were a few hyaline bodies without cytoid deposition. IgM and fibrinogen were found in all cases except one for IgM. Deposition of IgG was detected in four cases and intensity of the fluorescence appeared to be weaker than that for IgM and fibrinogen. Reactivity to IgA and complement was also detectable in a few cases. In addition, a linear deposit of both IgG and fibrinogen at the basement membrane was found in two cases and in three cases there was fibrinogen alone.

### *Malignant dermatoses*

In Bowen's disease, eosinophilic cells, demonstrating individual cell keratinization, were seen in the



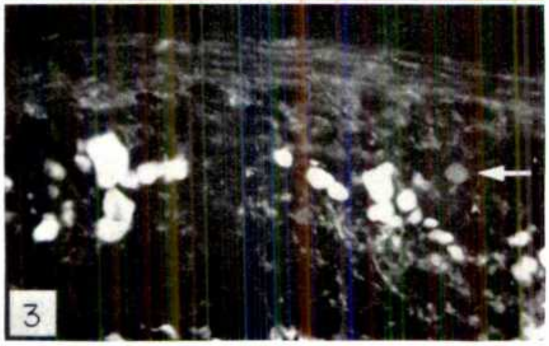


FIGURE 2. Erythema following UV light exposure, stained with FITC-labelled anti-human C3, demonstrating apparent fluorescence on sunburn cells (× 700).

FIGURE 3. Lichen planus, stained with FITC-labelled anti-human IgM. Brilliant fluorescence on subepidermal hyaline bodies was seen. A hyaline body was not associated with any deposition (arrow) (× 680).

hypertrophic epidermis. Comparison of the observations between light and fluorescence microscopy showed that no specific fluorescence for immunoglobulins, complement or fibrinogen was associated with these structures. Epidermal eosinophilic cells found in squamous cell carcinoma did not show any fluorescence for serum factors either (Fig. 4). In one case, a few fluorescent ovoid bodies showing the deposition of IgG and fibrinogen were noticed in the dermis. These bodies were positively stained with thioflavin T (Luna, 1968), suggesting that they may be amyloid-like structures.

## DISCUSSION

Deposition of immunoglobulins, complement and fibrinogen was detected on some of the eosinophilic cells present in the epidermis of Mucha-Habermann's disease and UV light-induced erythema. Deposition of these serum factors was more often found on subepidermal hyaline bodies in lichen planus. In contrast, no such deposits were demonstrated on eosinophilic cells of neoplastic dermatoses. Both benign eosinophilic cells, including subepidermal hyaline bodies in lichen planus, and neoplastic eosinophilic cells are considered to be of epidermal origin. Electron microscopy has shown an aggregated tonofilamentous structure in sunburn cells (Olson, Gaylor & Everett, 1974; Olson & Everett,

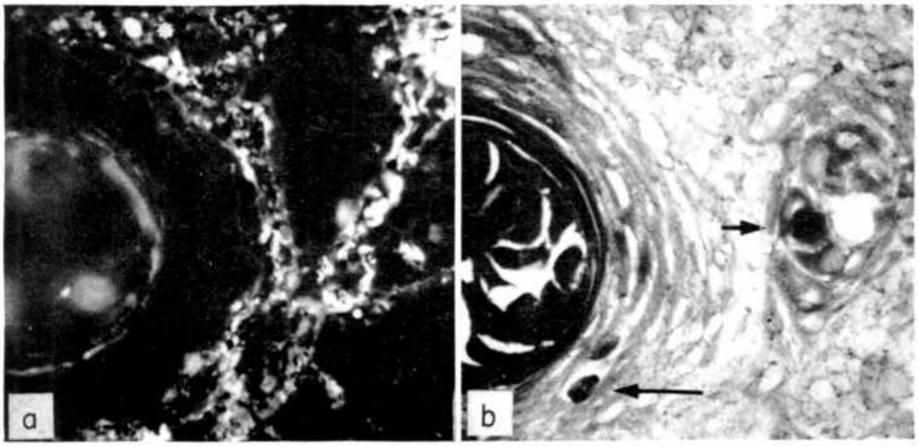


FIGURE 4. (a) Squamous cell carcinoma, stained with FITC-labelled anti-human IgG, illustrating the absence of deposition on individual cell keratinization (I.K.). No specific fluorescence was seen over the section except autofluorescence and a few fluorescent ovoid bodies present in the dermis (asterisk) (× 650). (b) The frozen section restained with H & E after immunofluorescence microscopy, demonstrating the presence of I.K. (arrow) and a horn pearl (× 650).

1975), subepidermal hyaline bodies in lichen planus (Eady & Cowan, 1978; Ebner & Gebhart, 1972; El-Labban, 1970; El-Labban & Kramer, 1974; Ishibashi, Tsuru & Kukita, 1978; Hashimoto, 1976) as well as neoplastic eosinophilic cells in Bowen's disease (Karásek *et al.*, 1971; Olson, Nordquist & Everett, 1969) and squamous cell carcinoma (Hashimoto, 1976). However, qualitative findings of immunofluorescence microscopy were different in benign and neoplastic conditions.

Two hypotheses may be raised to explain the reason why serum factor deposition was associated with benign eosinophilic cells but not with neoplastic eosinophilic cells. Firstly, the formation of benign eosinophilic cells may be mediated by serum factor deposition, whereas neoplastic eosinophilic cells could be the result of somewhat different mechanisms. An alternative explanation is that the fluorescence detected on benign eosinophilic cells represents the secondary involvement of serum proteins rather than any immunological reaction and that absence of deposition on neoplastic eosinophilic cells merely results from the absence of penetration by serum proteins into the epidermis.

Our immunopathological findings in benign dermatoses seem to favour the latter possibility. Deposits on sunburn cells are probably not responsible for their formation since sunburn cells appear immediately after UV light exposure (Woodcock & Magnus, 1976). Deposition of serum proteins in Mucha-Habermann's disease was associated with only a limited number of eosinophilic cells. Some cells were free from deposits, suggesting that eosinophilic cell formation in this disease may occur without deposition of humoral factors.

Major deposits on subepidermal hyaline bodies in lichen planus were IgM and fibrinogen. Fluorescence for IgG, IgA and C1q was less conspicuous. These findings are consistent with those reported by others (Baart de la Faille-Kuyper & Baart de la Faille, 1974; Salo *et al.*, 1972). It is assumed by these investigators that deposition of fibrin and IgM with complement is essential to hyaline body formation. However, our observation that there were hyaline bodies without serum factor deposition (although they were not numerous) may argue against this view. Abell *et al.* (1975) and Michel & Sy (1973) considered these deposits a non-specific phenomenon.

Recently, a concept termed 'apoptosis' has been proposed as a cell deletion mechanism (Kerr, Wyllie & Currie, 1972; Kerr & Searle, 1972). Eosinophilic or dyskeratotic cells are considered to represent a kind of apoptosis occurring in the epidermis (Hashimoto, 1976; Olson & Everett, 1975). The possibility that serum factor deposition on eosinophilic cells is taking part in apoptosis remains to be studied.

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# Erythema multiforme: demonstration of immune complexes in the sera and skin lesions

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## SUMMARY

In twenty patients with erythema multiforme we investigated circulating immune complexes and their deposition in the skin lesions. C1q-binding activity was elevated in ten of twenty patients, and the platelet aggregation titre was high in three of twelve tested sera. Decreased levels of C3 were seen in two and of C4 in one out of eighteen patients. Direct immunofluorescence showed a deposition of C3, IgM or IgG in the blood vessel walls of the upper dermis in four of twelve patients. These findings may suggest that transient production of circulating immune complexes and their deposition play an important role in the pathogenesis of this disease.

Erythema multiforme is a distinctive skin disease, characterized by a sudden onset of round erythematous macules, papules, vesicles or bullae appearing symmetrically over the hands and extensor aspects of the extremities and less commonly over the trunk. Systemic symptoms are usually minimal, but severe complications do occur. Various agents such as herpes simplex virus, mycoplasma pneumoniae, streptococcus or drugs are reportedly involved as causes of this disease. Although immune mechanisms have been speculated, the details remain obscure.

Recently, the deposition of immunoglobulins and complement in the blood vessel walls of the early lesions of this disease (Kazmierowski & Wuepper, 1978) and presence of immune complexes in the circulation (Swinehart *et al.*, 1978; Yanase & Imamura, 1979) have been reported.

This investigation was designed to make a simultaneous demonstration of circulating immune complexes and their deposition in the skin lesions of twenty patients with erythema multiforme.

## MATERIALS AND METHODS

Twenty patients with erythema multiforme were investigated. Seventeen patients had the lesions on the hands. In no patient were drugs considered to be the cause. Biopsy specimens were obtained

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from early lesions and divided into two. One specimen was fixed in 10% formalin for routine haematoxylin and eosin staining, while the other was quick-frozen in an acetone-dry ice mixture and stored until use. Six micron sections were cut in a cryostat at −20°C and stained with FITC-labelled anti-human IgG, IgM, IgA, C3, C1q and fibrinogen. Protein concentrations and F/P molar ratio of the antisera were: 4·4 mg/ml, 1·74 for anti-human IgG; 3·5 mg/ml, 1·94 for anti-human IgM; 4·1 mg/ml, 1·40 for anti-human IgA; 2·5 mg/ml, 1·54 for anti-human C3; 4·4 mg/ml, 2·12 for anti-human C1q; and 4·1 mg/ml, 2·55 for anti-human fibrinogen, respectively. All conjugates were diluted to 1:10 with 0·01 M phosphate buffered saline (pH 7·2). Sections were examined under a Nikon epi-illumination fluorescence microscope. Photographs were taken on Fuji colour reversal film.

### *Serum samples*

Serum samples were collected simultaneously with the skin biopsy specimens. Whole blood was drawn, allowed to clot at room temperature, and the serum was stored at −80°C until use.

*C1q-binding test.* Two milligrams of highly purified C1q was isolated from 250 ml of normal human blood by the method of Yonemasu & Stroud (1971). After radio-iodination of C1q with lactoperoxidase, the C1q-binding test was performed according to the method of Zubler *et al.* (1976). Briefly, 50 μl of tested sera were incubated with 100 μl of 0·2 M EDTA (pH 7·5) for 30 min at 37°C to prevent the integration of $^{125}$IC1q into the intrinsic C1qrs complex. Then 50 μl of $^{125}$IC1q diluted with 10% BSA-veronal buffered saline, and 3% polyethylene glycol were added to each duplicated tube (Shionogi-polystyrene tube, Japan) and incubated for 60 min in an ice bath. Free C1q was removed from C1q bound to complex by centrifugation and radioactivity in the precipitates was measured. Results were expressed as per cent $^{125}$IC1q precipitated as compared with the radioactivity precipitated in a 15% trichloroacetic acid control tube. C1q-binding activity (C1q-BA) of twenty-four normal control sera was less than 21·4% (Fig. 1).

*Platelet aggregation test.* Platelet aggregation test was performed as described by Penttinen *et al.* with slight modifications (Yanase & Imamura, 1979). Platelet aggregation titre (PA titre) of 23; normal control sera was less than 8.

*Determination of complement.* Protein concentrations of C3 and C4 were determined by single radial immunodiffusion, using commercial plates (Hoechst). Normal ranges of C3 and C4 were 64–116 mg/dl and 17–49 mg/dl, respectively.

## RESULTS

The results of C1q-BA, PA titre, concentrations of C3 and C4, and the direct immunofluorescence are shown in Table 1.

In ten of twenty patients, C1q-BA was higher than that of the control sera (Fig. 1). However, the PA titre was high in only three of twelve tested sera. Two of these three sera showed high C1q-BA, while C1q-BA in the other serum was within normal range.

Levels of complement were almost within normal limits. In only two out of eighteen patients was the level of C3 slightly decreased, and one of them was associated with a slight decrease in C4. In these two patients, C1q-BA was elevated.

Direct immunofluorescence showed deposition of immunoglobulins and/or complement in the blood vessel walls of the upper dermis in four of twelve patients (Figs 2–4). C3 was deposited granu-

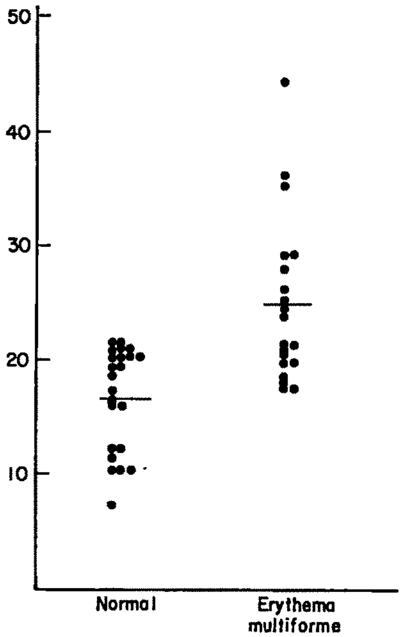


FIGURE 1. C1q-BA in the sera of normal subjects and patients with erythema multiforme. Mean ± s.d. for normal subjects = 16·3 ± 4·4; for erythema multiforme patients = 26·4 ± 7·0.

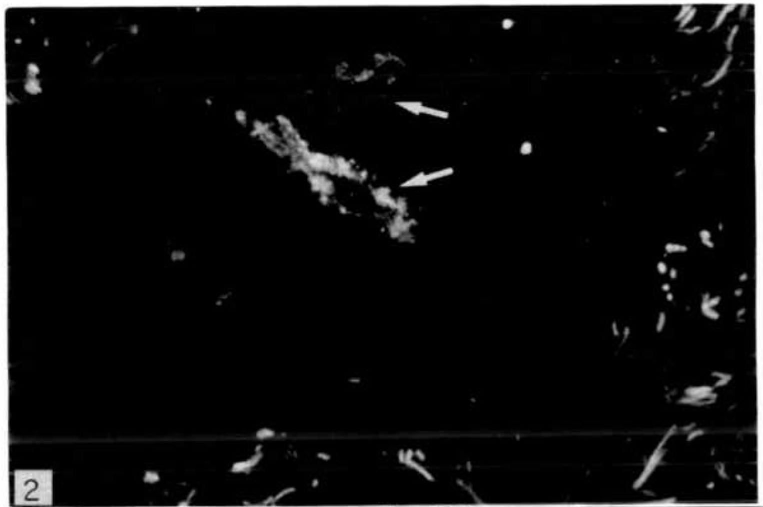
larly in three patients, in one of whom IgM deposition was associated. IgG deposition was found in one patient. Elevated C1q-BA was noted in one of four patients who showed positive immunofluorescence.

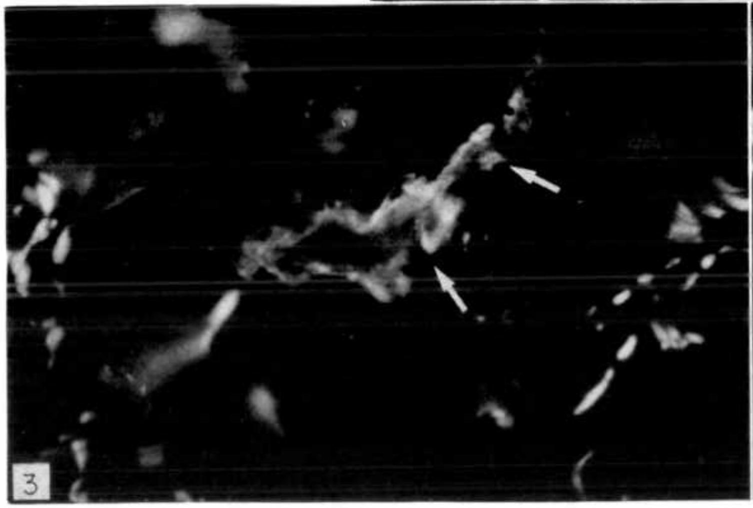
## DISCUSSION

C1q-BA was elevated in the sera from half the patients with erythema multiforme. In addition, some of the sera showed a high PA titre. Direct immunofluorescence revealed deposition of immunoglobulins and complement, mostly in a granular pattern, in the blood vessel walls in the upper dermis of the lesions, though it was seen in only a limited number of patients. These findings are consistent with recent reports on the presence of cryoimmunoglobulins and abnormal C1q-binding (Swinehart *et al.*, 1978) and high PA titre (Yanase & Imamura, 1979) in the sera and on the deposition in the skin tissues (Kazmierowski & Wuepper, 1978). Although both C1q-binding test and platelet aggregation test detect aggregated immunoglobulins as well as immune complexes, these data strongly suggest that the development of this disease is mediated by the immune complexes.

Failure to demonstrate C1q-binding materials, platelet aggregation materials or a decreased level of complement in a considerable number of sera or deposition of immunoglobulins or complement in a majority of skin specimens and lack of correlation between them may be due to the transient production and deposition of immune complexes in this disease. Kazmierowski & Wuepper (1978) succeeded in demonstrating the deposition of IgM and C3 only in the early lesions less than 24 h old.

Recently, it has been suggested that cutaneous necrotizing vasculitis which histologically shows fibrinoid degeneration of the blood vessel walls and accumulation of inflammatory infiltrates around them, with a predominance of neutrophils, is induced by immune complexes. The presence of circulating immune complexes (Asghar, Faber & Cormane, 1975; Braun-Falco, Männel & Scherer, 1977;





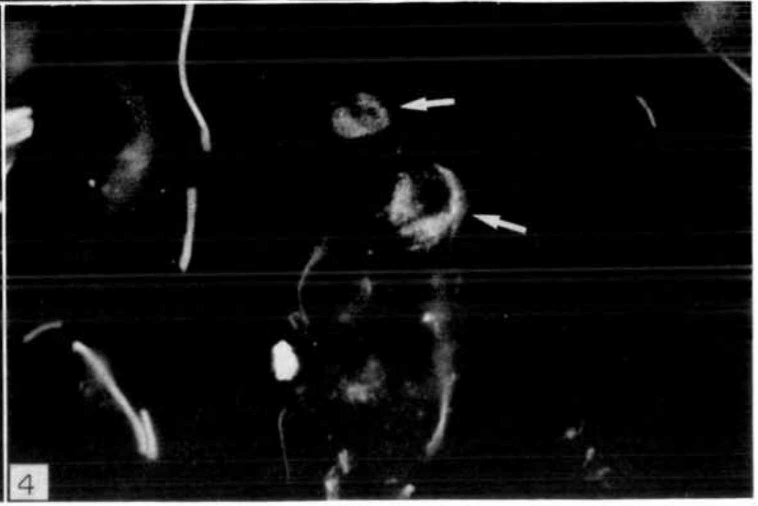


FIGURE 2. Case 1. Deposition of C3 in blood vessel walls in the upper dermis (arrows) (× 270).
FIGURE 3. Case 1. Deposition of IgM in a blood vessel wall in the upper dermis (arrows) (× 450).
FIGURE 4. Case 2. Deposition of IgG in blood vessel walls in the upper dermis (arrows) (× 550).

TABLE 1. C1q-BA, PA titre and protein concentrations of C3 and C4 in the sera and direct immunofluorescence in the skin lesions

| Patient | Age | Sex | Site of lesions | C1q-BA (%) | PA titre | C3 (mg/dl) | C4 (mg/dl) | Direct immunofluorescence |
|---|---|---|---|---|---|---|---|---|
| 1. M.K. | 28 | F | Palms, upper arms, knees | 19·4 | 8 | 143·0 | 55·8 | M(+), C3(+) |
| 2. I.N. | 32 | M | Dorsa of hands and feet, elbows, knees | 17·6 | 16 | 97·0 | 45·0 | G(+) |
| 3. S.S. | 29 | M | Dorsa of hands | 35·0 | 8 | 104·0 | 31·5 | n.d. |
| 4. H.N. | 45 | F | Dorsa of hands, extensor surfaces of extremities | 29·0 | n.d. | 40·0 | 15·0 | (−) |
| 5. K.N. | 27 | M | Forearms, extensor surfaces of lower extremities | 20·7 | 2 | 98·0 | 58·3 | n.d. |
| 6. K.N. | 25 | M | Dorsa of hands, extremities | 17·2 | 2 | 93·5 | 33·5 | (−) |
| 7. M.T. | 31 | F | Dorsa of hands | 17·3 | 4 | 90·0 | 38·3 | (−) |
| 8. Y.Y. | 23 | F | Dorsa of hands and feet, extensor surfaces of extremities | 20·1 | n.d. | 79·0 | 24·5 | n.d. |
| 9. F.Y. | 47 | F | Dorsa of hands and feet | 26·0 | (−) | 77·5 | 49·5 | C3 (+) |
| 10. H.S. | 24 | F | Dorsa of hands | 44·1 | 4 | n.d. | n.d. | (−) |
| 11. T.H. | 66 | M | Upper arms, face, trunk | 25·1 | n.d. | 93·5 | 36·0 | (−) |
| 12. Y.K. | 36 | F | Dorsa of hands, extensor surfaces of extremities | 29·0 | n.d. | 108·0 | 37·0 | (−) |
| 13. Y.K. | 45 | M | Abdominal wall | 19·2 | 8 | 64·0 | 21·8 | n.d. |
| 14. T.N. | 7 | M | Dorsa of hands, trunk, extremities | 24·4 | n.d. | 80·0 | 73·0 | n.d. |
| 15. Y.K. | 36 | F | Dorsa of hands, elbows, knees | 21·1 | n.d. | 66·0 | 47·5 | C3 (+) |
| 16. Y.A. | 24 | F | Dorsa of hands, forearms, face | 23·4 | n.d. | 56·0 | 34·5 | (−) |
| 17. N.M. | 6 | F | Dorsa of hands, forearms, thigh, abdominal wall | 18·2 | n.d. | 71·5 | 48·3 | (−) |
| 18. K.T. | 20 | F | Fingers | 27·7 | 16 | 89·0 | 23·5 | n.d. |
| 19. H.T. | 27 | F | Dorsa of hands | 36·3 | 128 | n.d. | n.d. | n.d. |
| 20. S.F. | 54 | M | Dorsa of hands, extremities, chest | 20·9 | 2 | 76·0 | 35·5 | n.d. |

n.d. = not done.

Yanase & Imamura, 1979), deposition of immunoglobulins and complement in the blood vessel walls (Schroeter *et al.*, 1971; Sams *et al.*, 1975) and reproduction of skin lesions by the injection of histamine (Braverman & Yen, 1975; Wolff *et al.*, 1978) or sera (Jones *et al.*, 1976) all support this hypothesis.

On the other hand, Clayton *et al.* (1977) and Clayton & Hattenden (1978) reported the presence of circulating immune complexes and deposition of immunoglobulins and complement in the dermal vessels in the majority of patients with pityriasis lichenoides, in which histology reveals lymphocytic infiltration around the blood vessels.

Erythema multiforme is histologically characterized by a perivascular lymphocytic infiltration in the upper dermis. However, in some cases, a small number of neutrophils is associated with lymphocytes, and endothelial swelling or thickening of small vessel is seen (Graham & MacVicar, 1972; Lever & Schaumburg-Lever, 1975). In some of the present cases, nuclear dust was seen around the slightly thickened blood vessel walls. These findings may suggest that erythema multiforme is an immunological disease of a type intermediate between necrotizing vasculitis and pityriasis lichenoides and that the transient production of immune complexes plays an important role in the pathogenesis of this disease.

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# Studies on fibronectin in the skin

## IV. INDIRECT IMMUNOFLUORESCENCE STUDIES IN LUPUS ERYTHEMATOSUS

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### SUMMARY

Fibronectin is an important constituent of normal human skin, mediating cell/cell and cell/fibre interactions. In affected skin in discoid and systemic LE, changes in the distribution of fibronectin in the dermo–epidermal junction and in the papillary dermis are observed with homogenization and degenerative changes, IF negative gaps and slit formation in the dermo–epidermal region, together with IF positive globular bodies and transport of fibronectin into the epidermis. Unaffected skin in LE demonstrates the pattern of fibronectin as found in normal human skin.

Fibronectin is a normal glycoprotein of the human body. It exists in two forms: (1) as plasma fibronectin, also called cold-insoluble globulin (Mosesson & Umfleet, 1970); and (2) as cellular fibronectin, produced mainly by fibroblasts (Ruoslahti & Vaheri, 1975) and constituting an important component of the connective tissue. Fibronectin has a special affinity to collagen (Kleinmann *et al.*, 1978), and *in vitro* studies demonstrate adhesional and spreading properties (Klebe, 1974). In the skin, fibronectin is found mainly in structures rich in collagen, mostly at the dermo–epidermal junction and in the dermis (Fyrand, 1979a). Changes in the normal distribution of fibronectin have been demonstrated in psoriasis vulgaris (Fyrand, 1979b) and in lichen planus (Fyrand, 1979c). In the present study the distribution of fibronectin in the skin in lupus erythematosus is described.

### MATERIAL AND METHODS

Biopsies were taken from clinically affected and unaffected skin from twelve patients (five women and seven men) with lupus erythematosus (LE). The diagnosis was based upon the clinical picture, serological investigations and skin biopsies examined with light microscopy and direct immunofluorescence (IF) techniques. Two of the patients had systemic LE and ten patients had chronic discoid LE.

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The skin samples were investigated with the indirect IF technique using a specific antiserum against fibronectin. Further details of biopsies, IF technique, microscopy and microphotography are described elsewhere (Fyrand, 1979a).

## RESULTS

### *Unaffected skin*

The same IF-pattern of fibronectin is observed as in normal human skin (Fig. 1).

### *Affected skin*

*Dermo–epidermal junction (DEJ)*. In the clinically unaffected part of the sections the normal distribution of fibronectin is observed but, when moving into the affected area of the sections, gradual changes in the distribution of fibronectin are found. In all samples examined, the continuous IF pattern of the DEJ becomes thicker, homogenized and hazy. In most sections this is followed by degenerative changes and IF negative gaps. The structure of the DEJ seems to dissolve and diffuse into adjacent epidermal areas (Fig. 2a) together with a parallel decrease of the IF intensity. Normally the IF pattern allows a clear distinction between epidermal and dermal components, but on account of degenerative changes of the DEJ this is impossible in some parts of the sections.

IF positive material often forms globular structures located in the DEJ or in the adjacent epidermal or dermal structures (Fig. 2b). In some sections, slit formation is found between the DEJ and the epidermis. The IF positive material is always found at the bottom of the slit.

*Epidermis*. In the unaffected part of the sections, the normal palisade arrangement of the basal cell layer is observed. In this part of the epidermis no IF is observed apart from a slight autofluorescence of the stratum corneum also present in control sections. In the affected areas of the sections the

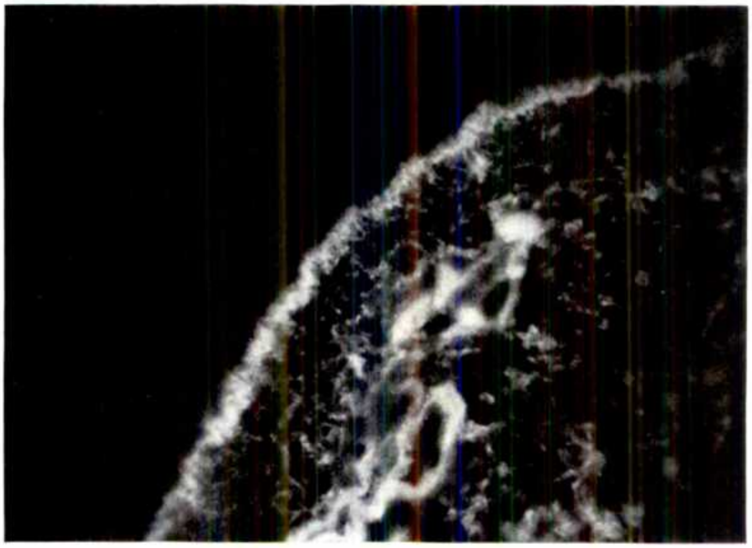

FIGURE 1. Fibronectin in unaffected skin in LE (× 360).

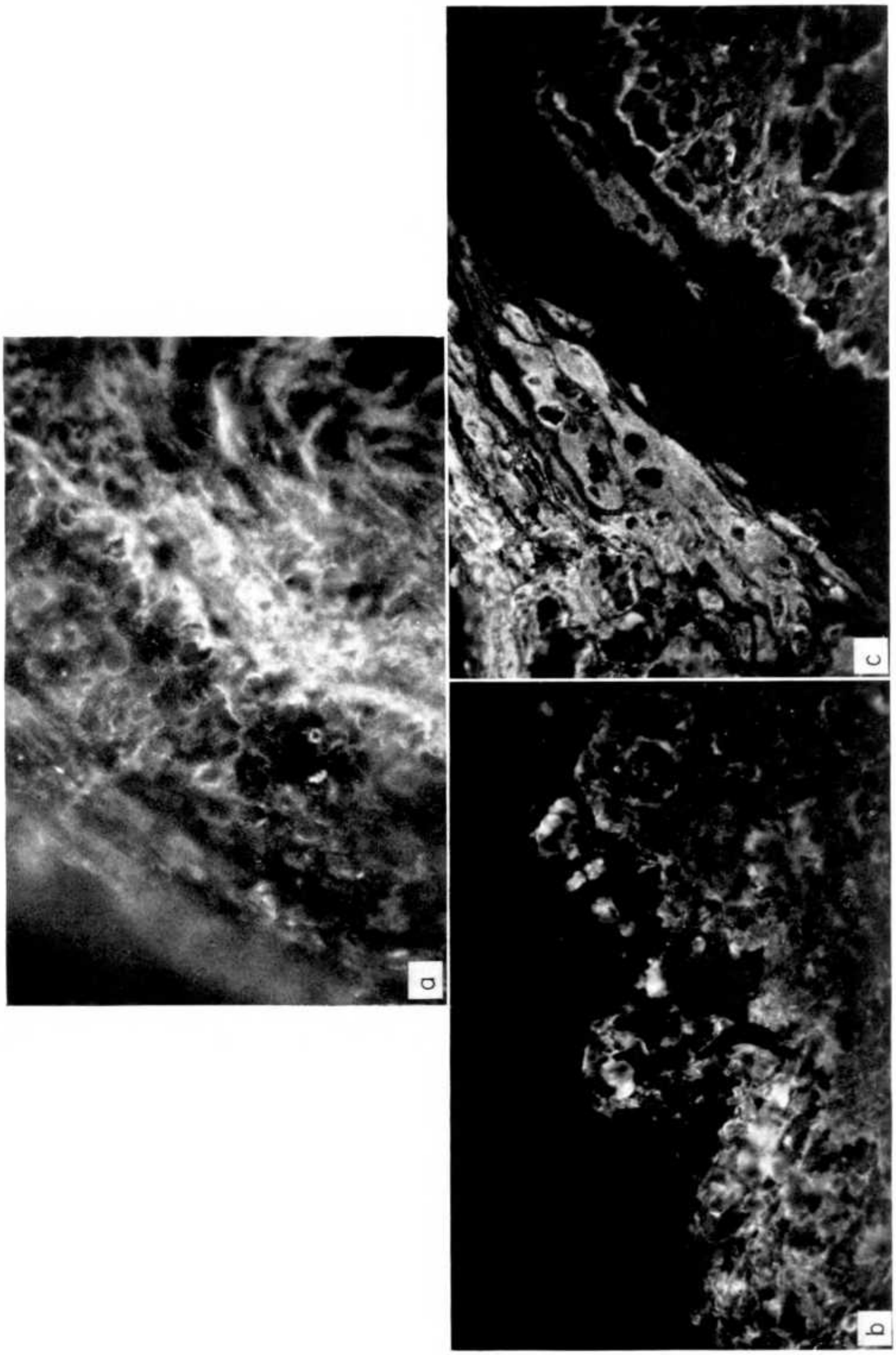

FIGURE 2. Fibronectin in affected skin in L.E. (a) Disintegration of the dermo-epidermal junction and the upper part of the papillary dermis with transport of fibronectin into the epidermis (× 320). (b) Formation of globular bodies with fibronectin in the disintegrated dermo-epidermal junction area together with swelling and homogeniaztion of the fibroncectin-containing structures in dermis (× 200). (c) Deposition of fibronectin in the upper part of the epidermis (× 200).

palisade arrangement of the basal cell layer is lost together with changes in the DEJ and in the papillary dermis. With increasing changes of the DEJ and the papillary dermis, IF positive material is now found in epidermal structures (Fig. 2a, c) with a low concentration in the lower epidermal layers, but increased in the upper layers (Fig. 2c). This IF is located inter- and intracellularly, mostly with a diffuse cytoplasmic staining pattern leaving a nucleus-like round central structure free from IF (Fig. 2c). In two of the cases examined, the cellular structure of these areas is lost resulting in diffuse and homogenous IF positive material.

*Dermis.* The major changes in the dermal distribution of fibronectin are observed in the papillary dermis. In the less affected part of the sections, the normal reticular structure of fibronectin is observed. In the affected areas, changes in the distribution of fibronectin are found together with changes in the DEJ, resulting in increased intensity of the IF staining. The reticular structure of fibronectin becomes thicker and homogenous up to a point when degradation and dissolving is observed. At this stage the IF staining intensity is reduced in the DEJ and in the dermis (Fig. 2b), resulting in loss of the outline of dermal structure with parallel disintegration of epidermal, dermo-epidermal and dermal structures. In these areas transport of IF positive material into the epidermis is found. These dermal changes affect mainly the papillary dermis, together with oedema and inflammatory changes. In the reticular dermis a moderate increase of the fibrillar staining is observed, but without degenerative changes.

## DISCUSSION

The involvement of the skin in systemic or discoid LE results in a variety of clinical manifestations from erythema and hyperkeratotic lesions to chronic atrophic scarring. This correlates with the variety of histological manifestations which are dependent upon the type and the duration of the disease. In most cases focal liquefaction degeneration of the epidermal basal cell layer is the most significant histological feature in LE, together with oedema and inflammatory infiltration in the dermis and deposition of immunoglobulins and complement in the junction area (Cormane & Jost, 1973).

In normal skin a characteristic pattern of fibronectin is observed, mainly at the DEJ and in the papillary dermis. In the DEJ fibronectin forms a continuous linear band, and in the papillary dermis a reticular structure is observed. In LE, these patterns are observed in unaffected skin together with the normal palisade arrangement of the basal cell layer of the epidermis. In the lesion, the palisade structure of the stratum basale is lost together with gradual IF changes in the DEJ and the dermal areas. In the DEJ the structure of fibronectin becomes broader, homogenized and increasingly hazy, resulting in a destructive degeneration of fibronectin in the DEJ and the dermal areas. By this process IF negative gaps are formed, probably representing Civatte bodies. Civatte bodies are believed to originate from the epidermal basal cell layer (Thyresson & Moberger, 1957). In the present study the IF positive material in these globular structures is derived from the DEJ and possibly from dermal structures, and not from the epidermal cell layer which is free from fibronectin, at least in the areas not adjacent to the basement membrane. In cultures of basal epithelial cells able to differentiate into epidermal cells, fibronectin is not demonstrated (Sun & Chen, as cited in Chen, Gallimore & McDougall, 1976). In lichen planus, similar globular structures are more abundantly found (Fyrand, 1979c). The slit formation observed in some sections is probably the result of degenerating epidermal basal cells, and the fibronectin-containing matrix is always found at the bottom of the slit. Adhesional properties of fibronectin have been demonstrated in fibroblast cell cultures (Klebe, 1974), and fibronectin mediates contact with collagen (Kleinmann *et al.*, 1978). Slit formation as an incipient bullous reaction is found only in areas with degeneration of fibronectin in the DEJ, but whether or not fibronectin is a factor responsible for epidermal adhesion remains to be investigated. Together with the findings in the DEJ, changes of the fibronectin pattern in the dermis are observed, mostly in the upper part of the dermis. This results in degeneration and dissolving of fibronectin structures and in transport of fibronectin into the epidermis. In some of our cases this is a prominent feature, especially in hyperkeratotic types of LE. Whether or not this is an active or a passive transport, and if this protein has a pathogenetic influence on the development of LE lesions, is uncertain. Fibronectin is demonstrated mainly intracellularly in epidermis. In LE, this may be one way of the disposing of degenerated material from the DEJ and the dermis. However, in lichen planus an intraepidermal transport of fibronectin is not observed, although the IF picture is very similar to that observed in LE (Fyrand, 1979c). In psoriasis, deposition of fibronectin is regularly observed in the stratum corneum area, localized mainly above elongated dermal papillae. This is possibly based on exoserosis of plasma from the capillary loops and the IF material may therefore represent circulating cold-insoluble globulin, also called plasma fibronectin, possibly representing a pathogenetic aspect different from that in LE (Fyrand, 1979b).

The significance of these changes in the normal distribution of fibronectin in the skin in LE is unknown.

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# Immunological aspects of psoriasis

## IV. PRESENCE OF CIRCULATING IMMUNE COMPLEXES IN PATIENTS BEFORE AND AFTER PUVA THERAPY; CORRELATIONS WITH T-CELL MARKERS

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### SUMMARY

We investigated sixteen patients before and after 1 month of PUVA therapy (three times a week) for circulating immune complexes (CIC) and T-cell markers.

CIC were detected by the PEG-C4 assay, using a laser nephelometry determination of polyethylene glycol (PEG, 3·5%) precipitated endogenous C4. T cells were evaluated by E rosettes, active E rosettes, anti-HTLA serum and ox EA $\gamma$-rosettes. This latter method investigated mainly T cells bearing Fc receptors for IgG (T$\gamma$-cells).

After 1 month of PUVA-therapy: (a) the cutaneous lesions markedly improved; (b) the mean percentages of E rosettes, active E rosettes, and HTLA bearing cells, depressed before treatment, returned to normal; (c) CIC were found in higher amounts; (d) T $\gamma$-cell numbers decreased simultaneously.

The improvement of T-cell levels assessed by E rosettes, active E rosettes and HTLA values was correlated with clearing of skin lesions, as previously reported by others. However, the increase of CIC during PUVA therapy, their correlation with T $\gamma$-cell decrease, and their possible role in the pathogenic chain of psoriasis remains uncertain.

Immunological factors in psoriasis have been reviewed in a previous paper (Guilhou, Clot & Meynadier, 1978). In the epidermis immunoglobulins, complement components and anti IgG factors (Krogh & Tonder, 1973) have been demonstrated. Immunoglobulin and complement deposits have also been found in dermal vessels (Ullman *et al.* 1978). Furthermore we and others (Guilhou, Meynadier & Clot, 1973; Florin-Christensen *et al.*, 1974) have reported circulating anti-IgG factors.

Such features are often associated with circulating immune complexes (CIC) in other pathological conditions (McDuffie, 1978) where they are involved in the genesis of the lesions. Moreover CIC could possibly interfere with lymphocyte membrane properties (Cordier, Samarut & Revillard, 1977).

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In this study CIC were found in patients before PUVA therapy. They increased after 1 month's treatment and were found to be correlated with modification of lymphocyte subsets.

## MATERIALS AND METHODS

### *Patients*

Sixty-one patients with psoriasis were studied. There were forty males and twenty-one females (mean age = $45{\cdot}7 \pm 2{\cdot}3$; range = 15–80 years). The duration of the disease was from 4 months to 30 years. Of these patients, nineteen had few lesions, twenty-three had numerous disseminated lesions, and nineteen had generalized psoriasis. An arthropathy was noted in eight cases.

The patients in this study were separated into two groups: the first group before treatment, the second after 1 month's PUVA therapy. Sixteen of these patients studied before PUVA therapy were re-investigated again 1 month later for CIC and T-cell markers.

In most of the patients, skin lesions were active before therapy and inactive after 1 month's treatment.

The control group included fifty age-matched healthy subjects.

### *Determination of circulating immune complexes (CIC)*

Eight-four sera were obtained from the psoriatic patients. They were collected after a 12 h fasting period and immediately frozen at −80°C.

The CIC were studied by the technique proposed by Digeon *et al.* (1977), using the precipitation by polyethylene glycol (PEG) and the determination of complement component C4 in the precipitates (PEG-C4). Briefly, serum was diluted 1:25 in borate buffer and mixed with an equal volume of PEG (PEG 6000, Fluka) at a final PEG concentration of 3·5%. After 18 h incubation at 4°C, the mixture was centrifuged for 20 min at 20,000 *g*. The precipitate was then washed and resuspended in 200 μg of 0·9% NaCl.

The levels of C4 in sera before PEG precipitation and in precipitates were estimated by laser nephelometry (Hyland Nephelometer, Travenol Laboratories). The results were expressed as mg/dl. The results of PEG-C4 assays were calculated according to the formula:

$$\text{precipitated C4 (\%)} = \frac{\text{mg/dl of C4 in precipitate}}{\text{mg/dl of C4 in serum}} \times 100$$

### *Study of T-cell membrane markers*

Peripheral blood mononuclear cells were obtained by centrifugation on a Ficoll-Hypaque gradient (Clot, Charmasson & Brochier, 1978).

Four different markers were used. E-rosette-forming cells (E-RFC), 'active' E-RFC, and human T-lymphocyte antigens (HTLA) were studied according to methods previously reported in detail (Clot *et al.*, 1978). We investigated peripheral blood mononuclear cells bearing receptors for the Fc part of IgG, using a rosette technique (Ferrarini *et al.*, 1975) with ox erythrocytes (OE) coated with subagglutinating amounts of specific rabbit IgG antibody (A). Such an OEA-rosette assay is known to detect mainly a T-cell subset bearing Fc γ-receptors (T γ-cells) (Ferrarini *et al.*, 1975).

### *Statistical studies*

Student's '*t*'-test was used for comparisons of means between the two groups of patients and the control group. For sixteen patients investigated before and after 1 month's therapy Student's '*t*'-test was performed on paired values from each patient.

TABLE 1. Circulating immune complexes in two groups of psoriatic patients

| | Precipitated C4 percentage (mean ± s.e. mean) | Mean range for $P$<0·01 | Per cent positive (> 17%) |
|---|---|---|---|
| (a) Controls (50) | 13·2 ± 1·1 | 10·3–16·2 | 8·0 |
| (b) Untreated psoriasis (43) | 23·2 ± 2·2 | 17·3–29·1 | 48·8 |
| (c) Treated psoriasis (41) | 31·5 ± 3·0 | 23·4–39·6 | 75·6 |
| Comparisons between | | Chi square | |
| by Student's '*t*'-test | a/b $P$<0·001 | test | a/b $P$<0·001 |
| | a/c $P$<0·0001 | | a/c $P$<0·0001 |
| | b/c $P$<0·05 | | b/c ns |

## RESULTS

### *CIC in psoriatic sera*

PEG-C4 assays were performed in three groups of sera: (a) fifty control sera; (b) forty-three sera from untreated psoriatic patients; and (c) forty-one sera from psoriatic patients after 1 month's PUVA therapy. The results are shown in Table 1.

In normal sera, the mean percentage of precipitated C4 was 13·2 ± 1·1 (s.e. mean). Since the mean range for $P$<0·01 was 10·3–16·2%, values higher than 17% were considered as abnormal levels of precipitated C4. Only 8% of control sera showed values higher than 17%.

In psoriatic sera significantly higher amounts of precipitated C4 were found in untreated patients (23·2 ± 2·2) and in treated patients (31·5 ± 30). The frequency of positive PEG-C4 assays was 48·8% in untreated patients and 75·6% in treated patients. It must be noticed that the values in the group of treated psoriasis (inactive disease) were higher than in the group of untreated patients (active disease). These data were not correlated with the extent of the skin involvement.

The values of precipitated C4 increased with age in six psoriatic patients over 70 years old (mean percentage = 41·3 ± 6·8). The same phenomenon was observed in six healthy subjects over 70 years

TABLE 2. Circulating immune complexes and T-cell markers in sixteen psoriatic patients tested before and after 1 month's PUVA therapy

| | Precipitated C4 percentage (mean ± s.e. mean) | E-RFC | HTLA | 'active' E-RFC | OEA-RFC |
|---|---|---|---|---|---|
| Controls (50) | 13·2 ± 1.1 | 71·0 ± 1·0 | 75·5 ± 0·8 | 24·0 ± 1·4 | 25·4 ± 1·3 |
| Psoriasis before (16) | 21·6 ± 2·6 | 62·9 ± 2·4 | 74·4 ± 2·3 | 15·6 ± 2·1 | 21·5 ± 1·9 |
| Psoriasis after (16) | 30·8 ± 4·3 | 73·6 ± 1·8 | 82·0 ± 1·8 | 28·6 ± 3·3 | 14·3 ± 1·6 |
| Student's t test on paired values | | | | | |
| D before-after | −13·3 | −10·5 | −6·8 | −10·1 | +9·2 |
| $P$ | <0·01 | <0·001 | <0·001 | <0·01 | <0·001 |

The results are expressed as mean values ± standard error of the mean. E-RFC = E-rosette-forming cells. HTLA = human T-lymphocyte antigen. OEA-RFC = E.A.-rosette-forming cells using ox erythrocytes. D = mean difference. ns = not significant.

old (mean percentage = $21 \cdot 7 \pm 5 \cdot 1$) but the difference between precipitated C4 in these psoriatics and controls remained significant ($P < 0 \cdot 05$).

### *Evolution of CIC and T-cell markers after PUVA therapy*

Sixteen psoriatic patients were studied before and after 1 month's PUVA therapy. The data (Table 2) were compared for each patient by Student's '*t*'-test on paired values obtained before and after this treatment. After 1 month's treatment there was: (a) a significant increase of CIC; (b) a significant increase of T lymphocytes detected by E-RFC, HTLA antiserum and 'active' E-RFC. The numbers of E-RFC and 'active' E-RFC were significantly lower in these patients before treatment than in the control group ($P < 0 \cdot 001$, and $P < 0 \cdot 01$ respectively); (c) conversely, the percentages of OEA-RFC, which were similar in the patients before treatment and the control group, decreased significantly after 1 month's PUVA therapy.

## DISCUSSION

The data reported in this study can be summarized as follows:

Before PUVA therapy: CIC were present in about 50% of psoriatics, T cells were depressed and Tγ-cells were in normal percentages, in agreement with our previous reports.

After 1 month's PUVA therapy: CIC were found in higher amounts, T cells returned to normal values, and Tγ-cells decreased simultaneously.

The presence of CIC in psoriasis has recently been reported by Braun-Falco, Mannel & Sherer (1977). These authors, as in our preliminary study (Clot *et al.*, 1979) and in the present work, have found more positive tests in inactive psoriasis than in patients with exanthematic psoriasis.

However, it must be stressed that the nature of the material precipitated by PEG remains uncertain since this technique, like others used to detect CIC, does not identify them directly (Lambert *et al.*, 1978). In any case, the positive data obtained by two different methods and the presence of IgG rheumatoid factors in psoriasis sera (Guilhou *et al.*, 1973) argue in favour of such CIC.

A new finding in this study is the increased amount of CIC after 1 month's PUVA therapy. The effect of PUVA treatment on the immunological status of psoriastic patients has been demonstrated by several studies. These have shown a decrease of DNA synthesis by circulating leukocytes immediately after irradiation (Kraemer & Weinstein, 1977), inhibition of the *in vitro* mitogenic response to PHA (Scherer, Kern & Braun-Falco, 1977), modifications of E rosettes (Cormane *et al.*, 1977; Bohnert *et al.*, 1977), inhibition of chemotaxis (Langner & Christophers, 1977) and changes in surface immunoglobulins of peripheral leukocytes (Cormane, Hunyadi & Hammerlink, 1976).

The explanation of the increased CIC after PUVA treatment is hypothetical. PUVA therapy could remove preformed CIC coated on peripheral blood leukocytes in dermal vessels or release immune complexes fixed in psoriatic epidermal lesions. But PUVA therapy could also induce epidermal or dermal antigenic changes responsible for antibody production and subsequent CIC formation.

Normalization of T-cell values after PUVA therapy, assessed by three different markers, cannot be attributed to this treatment by itself but is probably due to the clearing of cutaneous lesions. Glinski *et al.* (1977) have demonstrated that E-RFC are decreased only in the active stages of the disease and return to normal after different treatments. Normal levels of T cells appeared simultaneously with high amounts of CIC. Thus it is likely that CIC are not responsible for the low levels of E-RFC and HTLA in active psoriasis. Conversely, the percentage of Fc γ-receptor cells decreased at the same time as the CIC increased. It is well known that such CIC, as well as aggregated immunoglobulins, are able to block the Fc-receptors (Cordier *et al.*, 1977). A large part of these cells (Tγ) can be modulated *in vitro* as well as *in vivo* (Moretta *et al.*, 1977; Pilcher, Lum & Broder, 1978) and are involved in the regulation of the immune response. Thus this study clearly demonstrates that the apparently

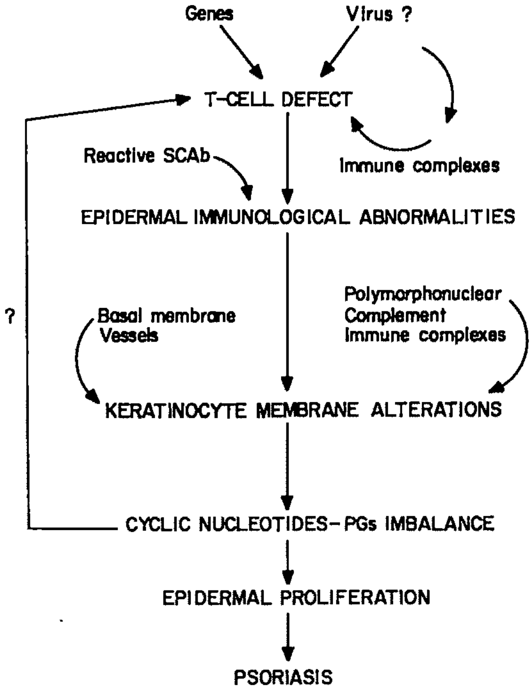

FIGURE 1. The possible involvement of circulating immune complexes in the pathogenic chain of psoriasis.

normal T-cell values (E rosettes) during the inactive stages of psoriasis cannot exclude impairment of some functional T-cell subsets.

In addition to their interaction with T-cell subpopulations, CIC could be implicated more directly in the skin lesions. They could react with epidermis and/or with polymorphonuclear leukocytes (Fig. 1). The presence of IC in psoriatic epidermis is strongly suggested by numerous epidermal immunological phenomena: presence of immunoglobulins and complement, rheumatoid factors (Krogh & Tonder, 1972), antinuclear antibodies (Cormane *et al.*, 1976) and fixed antistratum corneum antibodies (Beutner *et al.*, 1975). Moreover IgG and complement have recently been demonstrated in the walls of the dermal vessels (Ullman *et al.*, 1978). The fixation of complement on epidermal IC could result in polymorphonuclear attraction, enzyme release and keratinocyte membrane alterations (Orfanos *et al.*, 1973) leading to cell proliferation (van Scott & Ekel, 1963).

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# Psoriasis and HLA-Cw6

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## SUMMARY

The prevalence of histocompatibility antigen HLA-Cw6 was 72·7% in twenty-two patients with the guttate form and 45·9% in thirty-seven patients with the vulgaris form of psoriasis; the difference between these figures and the prevalence (7·4%) in 462 healthy blood donors in Finland is highly significant. The previously observed excess of several HLA-B locus genes might be secondary to their close linkage with the Cw6 gene. Hereditary factors appear more important in the guttate than in the vulgaris form of psoriasis.

The clear association of psoriasis with antigens controlled by the HLA gene complex has advanced the understanding of the disease as a polygenic and multifactorial condition (Karvonen, Tiilikainen & Lassus, 1976). Many HLA-B antigens are associated with it, but inconsistently in different populations.

Svejgaard *et al.* (1975) suggested that the multiple HLA-B antigen associations with psoriasis vulgaris might be secondary to an association of the disease with the HLA-C locus controlled antigen T7 which occurred in a positive genetic disequilibrium with the HLA specificities characteristic of psoriasis. They could not, however, prove this hypothesis, because at that time there was only one antiserum for T7, and in addition to anti T7, it also contained some other HLA antibodies.

The analysis of another anti T7 antiserum, which was found in our laboratory, was included in the Seventh International Histocompatibility Testing Workshop.

In the ensuing Conference (Oxford, 4–10 September 1977), the antigen was assigned the designation HLA-Cw6 (WHO-IUIS Terminology Committee Report, 1978), and it was indeed found to be increased in psoriatic patients (Tiilikainen *et al.*, 1977; Tsuji *et al.*, 1977).

This report describes the correlation between psoriasis and HLA-Cw6 in Finland.

## PATIENTS AND METHODS

### *Patients*

Forty unrelated patients, members of the Psoriasis League in Finland, volunteered to participate in

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the study described in a meeting of the League. They were interviewed and examined clinically, and the HLA antigens were determined as agreed by the participants of the Seventh International Histocompatibility Testing Workshop (Bodmer, 1978). Since the Workshop analysis suggested that patients with the guttate form of psoriasis might have more extreme alterations in the HLA antigen frequencies, the three patients of this type were separated from the above series. They were analysed together with a further series of nineteen unrelated subjects with guttate manifestations who attended an out-patient clinic specializing in PUVA treatment.

### *Controls*

The HLA phenotypes of random blood donors were determined by the same methods and the same local antisera as were used for the patients with psoriasis, but varying numbers of individuals were tested for different HLA-C and D specificities.

The Workshop antisera were not used in the analysis of blood donors or the additional series of patients with guttate psoriasis.

## RESULTS AND DISCUSSION

Table 1 presents the frequencies of HLA antigens in thirty-seven unrelated patients of the vulgaris type, those twenty-two with guttate psoriasis (including three of the forty League members), and blood donors.

HLA-B13 and B17 were increased in the patients, which is in good agreement with our earlier observations (Karvonen *et al.*, 1976). Five of the six patients with Cw2 were HLA-B27 positive as well; thus the frequent occurrence of Cw2 in the psoriatics was probably due to the presence of the *B27*, *Cw2* haplotype and hardly associated with psoriasis.

The marked increase of Bw4 in psoriasis (especially in the guttate form) is undoubtedly due to the high prevalence of B13, 17, 37 and Bw47.

HLA-Cw6 was found in seventeen of the thirty-seven patients with the vulgaris type of psoriasis. The incidence of 45·9% is significantly higher than the incidence (7·4%) among random blood donors ($\chi^2 = 55{\cdot}59$, $P < 0{\cdot}0001$ even after correction for the number of HLA specificities tested).

In the guttate form of psoriasis the incidence of Cw6 tends to be even higher than in psoriasis vulgaris; it was present in 72·7% (sixteen of twenty-two subjects). The difference from blood donors is even more impressive, with $\chi^2 = 96{\cdot}87$. However, guttate and vulgaris forms of psoriasis do not differ significantly from each other in the prevalence of Cw6 ($\chi^2 = 4{\cdot}01$).

To obtain an idea of the haplotypic associations of *Cw6*, the HLA-B phenotypes of all Cw6 positive psoriatics and blood donors are shown in Fig. 1. In both groups most subjects had either B13 or B17, and the distribution of phenotypes was very much the same. Thus there seems to be no *HLA-B,C* haplotype which would be characteristic of psoriasis. On the other hand, it is evident that a strong allelic association (or positive 'linkage disequilibrium') exists between *Cw6* and *B13*, *B17* (and *Bw47*) equally among blood donors and psoriatics. Each of these B locus specificities is more frequent in Fig. 1 than in Table 1, but the figures on both sides of the diagonal are well balanced (B13 was found in nineteen and B17 in ten of the thirty-three psoriatics, with B13 in eighteen and B17 in ten of the thirty-four blood donors). (However, the calculation of delta values to prove the strength of the allelic association falls out of the scope of this report.)

Among the twenty Cw6 negative patients with psoriasis vulgaris, B13 was observed twice, and B17 and B37 each once. The Bw16 subtype Bw38 was found once, and it was in a Cw6 negative patient. Of the six Cw6 negative patients with the guttate type, one had B13. Bw38 was found in one patient who was Cw6 positive. These figures are too low to justify any conclusion of the possible independent association between the HLA-B antigens and psoriasis.

TABLE 1. HLA antigens in vulgaris and guttate forms of psoriasis

| | | Psoriasis vulgaris | | Guttate psoriasis | | Blood donors | |
|---|---|---|---|---|---|---|---|
| Antigen | | % | Observations | % | Observations | % | Observations |
| A | 1 | 21·6 | (8/37) | 22·7 | (5/22) | 20·1 | (181/900) |
| | 2 | 73·0 | (27/37) | 77·3 | (17/22) | 54·0 | (486/900) |
| | 3 | 24·3 | (9/37) | 27·3 | (6/22) | 44·4 | (400/900) |
| | 9 | 24·3 | (9/37) | 9·1 | (2/22) | 16·2 | (146/900) |
| | 10 | 8·1 | (3/37) | 4·5 | (1/22) | 7·8 | (70/900) |
| | 11 | 5·4 | (2/37) | 9·1 | (2/22) | 8·8 | (79/900) |
| | 28 | 16·2 | (6/37) | 0 | (0/22) | 11·3 | (102/900) |
| | w19 | 8·1 | (3/37) | 27·3 | (6/22) | 19·6 | (176/900) |
| Unidentified | | | — | | — | | — |
| B | 5 | 8·1 | (3/37) | 4·5 | (1/22) | 11·9 | (107/900) |
| | 7 | 10·8 | (4/37) | 4·5 | (1/22) | 24·3 | (219/900) |
| | 8 | 5·4 | (2/37) | 4·5 | (1/22) | 20·0 | (180/900) |
| | 12 | 8·1 | (3/37) | 9·1 | (2/22) | 15·4 | (139/900) |
| | 13 | 35·1* | (13/37) | 40·9* | (9/22) | 6·0 | (54/900) |
| | 14 | 0 | (0/37) | 0 | (0/22) | 0·3 | (3/900) |
| | 15 | 27·0 | (10/37) | 18·2 | (4/22) | 20·1 | (181/900) |
| | 17 | 18·9 | (7/37) | 22·7 | (5/22) | 4·3 | (39/900) |
| | 18 | 8·1 | (3/37) | 0 | (0/22) | 10·0 | (90/900) |
| | 27 | 16·2 | (6/37) | 9·1 | (2/22) | 14·0 | (126/900) |
| | 37 | 2·7 | (1/37) | 4·5 | (1/22) | 1·6 | (14/900) |
| | 40, w41, w47 | 5·4 | (2/37) | 27·3 | (6/22) | 17·9 | (161/900) |
| | w16 | 18·9 | (7/37) | 22·7 | (5/22) | 9·3 | (84/900) |
| | w21 | 0 | (0/37) | 0 | (0/22) | 0·4 | (4/900) |
| | w22 | 10·8 | (4/37) | 13·6 | (3/22) | 5·0 | (45/900) |
| | w35 | 21·6 | (8/37) | 13·6 | (3/22) | 27·2 | (245/900) |
| Unidentified | | 0 | (0/37) | 0 | (0/22) | 0·3 | (3/900) |
| | w4 | 75·7 | (28/37) | 90·9 | (20/22) | 52·1 | (261/501) |
| | w6 | 81·1 | (30/37) | 86·4 | (19/22) | 88·6 | (444/501) |
| C | w1 | 13·5 | (5/37) | 18·2 | (4f22) | 14·3 | (69/483) |
| | w2 | 16·2 | (6/37) | 0 | (0/22) | 7·7 | (32/418) |
| | w3 | 32·4 | (12/37) | 18·2 | (4/22) | 32·1 | (155/483) |
| | w4 | 27·0 | (10/37) | 18·2 | (4/22) | 19·2 | (65/338) |
| | w5 | 5·4 | (2/37) | 9·1 | (2/22) | 7·1 | (6/84) |
| | w6 | 45·9* | (17/37) | 72·7* | (16/22) | 7·4 | (34/462) |
| | Blank | | (2/37) | | (2/22) | | (131/384) |
| D | w1 | 16·7 | (3/18) | nd | | 20·8 | (16/77) |
| | w2 | 0 | (0/18) | nd | | 26·0 | (20/77) |
| | w3 | 27·8 | (5/18) | nd | | 24·7 | (19/77) |
| | w4 | 5·6 | (1/18) | nd | | 8·1 | (5/62) |
| | w5 | 6·3 | (1/16) | nd | | 0 | (0/10) |
| | w6 | 22·2 | (4/18) | nd | | 21·0 | (18/77) |
| | w7 | 27·8 | (5/18) | nd | | 20·0 | (3/15) |
| | w8 | 29·4 | (5/17) | nd | | 14·3 | (2/14) |
| | Blank | | (2/18) | nd | | | (11/60) |

* $P < 0·0001$.

nd = not determined.

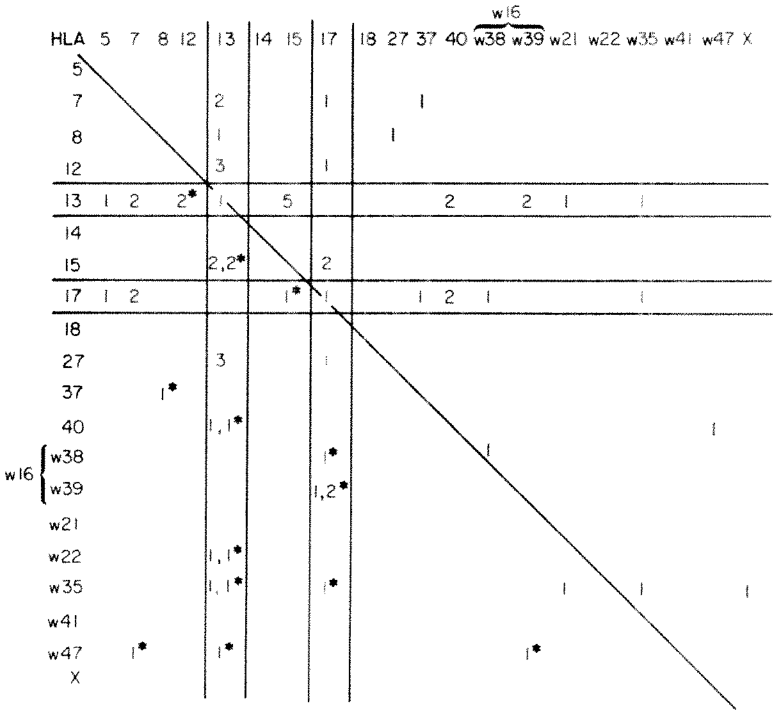

FIGURE 1. HLA-B specificities in HLA-Cw6 positive psoriatic patients and controls. Figures indicate numbers of subjects with the combination of B locus antigens defined by the intersecting horizontal and vertical line. Above the diagonal, distribution of the thirty-four Cw6 positive phenotypes in 462 blood donors; below the diagonal, seventeen phenotypes of thirty-seven patients with psoriasis vulgaris and sixteen phenotypes (indicated by asterisks) of twenty-two patients with guttate psoriasis. Homozygosity is probable in four subjects (all controls), once each for B13, 17, w38 and w35.

We conclude from the haplotype analysis above that the association of psoriasis with Cw6 is closer than with any of the HLA-B specificities known so far. Our few results with HLA-D specificities seem to suggest secondary haplotypic associations weaker than the correlation between the *HLA-C* locus and susceptibility to psoriasis.

It is clear that only a proportion of HLA-C specificities can be identified at present. In two patients with psoriasis vulgaris no HLA-C antigen was observed; in seven, Cw6 was found alone, but in only one instance with HLA-B antigens which, as is suggested by the figures in Fig. 1, may both occur in the same halotype with *Cw6*. In the guttate patients, four of sixteen subjects with Cw6 might be homozygous for the gene. Five possible homozygotes of 33 cannot be interpreted as a definite increase of *Cw6* homozygosity. Thus the data do not support a postulation that both HLA halotypes of an individual must provide a 'psoriasis susceptibility gene' for the disorder to be manifested.

The strength of the association between Cw6 and psoriasis vulgaris (i.e. the relative risk of a Cw6 positive subject developing the disease) calculated from Table 1 is 10·7. In guttate psoriasis it appears even higher, although the small number of patients makes such calculations irrelevant as yet.

The age at onset of psoriasis is lower among patients with a HLA-B 'marker' antigen (Karvonen, 1975), and thus it is conceivable that the same is observed with Cw6: mean age at onset in Cw6 carriers with psoriasis vulgaris was 21·3 ± 10·5 years, and in Cw6 negative patients, 31·3 ± 16·4 years. Corresponding ages in guttate psoriasis were: Cw6 positives, 13·0 ± 4·9 years, and Cw6 negatives, 34·8±17·8 years. These differences are almost significant for psoriasis vulgaris ($P<0{\cdot}02$) and are significant for guttate psoriasis ($P<0{\cdot}01$).

Patients with the guttate form more often had relatives with psoriasis (77·3%, or seventeen of twenty-two) than had those with the vulgaris form (43·2%, or sixteen of thirty-seven); this is almost significant ($\chi^2 = 6{\cdot}48$, $P<0{\cdot}02$). The distribution of family histories between Cw6 positives and negatives was roughly similar in the guttate group, but in the vulgaris group, 52·9% of the Cw6 positives (or nine of seventeen), had psoriatic relatives, as compared with 35% of the Cw6 negatives (or seven of twenty).

The accumulation of several hereditary aspects, documented by the simultaneous presence of Cw6 and family history, seems to lead to an early manifestation of the disease.

The findings in this small Finnish series of psoriasis patients are consistent with observations in other populations: the pooled results of the Seventh International Histocompatibility Testing Workshop (Batchelor & Morris, 1978) showed an excess of Cw6 in psoriasis, especially in the guttate form, and an early onset of the disease in bearers of this antigen. Thus, from the multiallelic associations between psoriasis and the HLA complex, it is now possible to discern one which is common to American and European Caucasians, Jews and Japanese. Interestingly, most of the other HLA associated diseases have the strongest associations with the *HLA-B* or *HLA-D* locus. Nearly all of these diseases are immunological, and because the hypothetical immune response (*Ir*) genes in man are postulated to be mainly in the B-D half of the HLA region, these HLA/disease associations may be explained by immune response regulation. On the other hand, the one well-established disease association of the *HLA-A* locus is with haemochromatosis, a clearly metabolic disease (Mackay, 1977; Simon *et al.*, 1977; Batchelor & Morris, 1978); neither is psoriasis a definitively immunological disorder. Thus, some disease susceptibility genes in the A-C half of the HLA region may regulate metabolic pathways that are independent of *Ir* genes. Whether or not the HLA associated psoriasis susceptibility gene in the proximity of the *HLA-C* locus has immune response regulating potential remains to be shown in the future.

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# Photochemotherapy of psoriasis: effects on bacteria and surface lipids in uninvolved skin

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## SUMMARY

The effect of PUVA therapy for psoriasis on the microbial flora and on levels and composition of skin surface lipid has been studied in ten patients. Samples were obtained from clinically uninvolved skin from the back and the forearm. There was no consistent significant change in the viable counts of aerobic cocci or anaerobic coryneforms during therapy. A transient rise in *Staphylococcus aureus* counts was thought to be due to the use of emollients rather than to an effect of PUVA. Genetic changes in the microorganisms were not sought.

There was an increase in the amount of total lipid (both casual levels and replacement sums) during therapy. An increase of free fatty acids at the expense of triglycerides did not reach statistical significance.

The influence of PUVA on the skin flora seems to be less *in vivo* than anticipated from *in vitro* studies.

Photochemotherapy with oral administration of psoralens and exposure to long wave ultraviolet irradiation (PUVA) is increasingly used for the therapy of various dermatoses. Above all its application for the treatment of psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1976) has shown an 'inflationary' trend. Due to the promising results and the enormous publicity this treatment is now used by numerous physicians without its possible adverse effects being sufficiently known.

The expansion of PUVA therapy and reports in the literature on the effects of 8-MOP and UV-A light on microorganisms *in vitro* caused us to consider the possible influence of such a potent pharmacophysical system upon the microbial ecology of human skin. We therefore examined psoriatic patients receiving photochemotherapy for possible changes in their resident skin flora and in their skin surface lipids, a factor which is of crucial importance in this microbial habitat.

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## MATERIALS AND METHODS

### *Patients and treatment*

Ten out-patients (four women, six men, average age 43 years, range 20–75 years) suffering from chronic plaque psoriasis and receiving photochemotherapy were studied. The treatment was carried out as described by Vella Briffa *et al.* (1978) and Rogers *et al.* (1979). Topical steroids or dithranol had been discontinued for at least 8 weeks prior to PUVA therapy in all except one case. The only topical treatment patients received during PUVA therapy was aqueous cream (B.P.) and emulsifying ointment (B.P.). However, patients were asked not to use any cream or ointment the day before the examination and to have a normal bath the night before the test.

Samples for skin bacteria and lipids were taken 2 h prior to irradiation on three occasions:

(1) Before the beginning of the treatment course.

(2) After six treatments (15 ± 4 $J/cm^2$).

(3) At the end of the treatment, when eight patients were considered clinically clear and two almost clear (18 ± 4 treatments; 85 ± 57 $J/cm^2$).

### *Sampling techniques*

Bacteriological samples were taken from clinically uninvolved skin of the posterior side of the forearm and of the interscapular region (T 3–4 midline) by the detergent scrub method (Williamson & Kligman, 1965) using 5 ml phosphate buffer pH 8 containing 1% Tween 80 with a Teflon cup and rod. Lipid samples were obtained in the direct vicinity of the bacteria sample site using a modification of the 'cup solvent method' (Herrmann & Prose, 1951; Greene *et al.*, 1970). Four millilitres of hexane/acetone 2:1 containing 25 $\mu$g/ml methyl nervonate as an internal standard were poured into a glass cylinder firmly held to the skin. The solvent was then stirred with a teflon spatula for exactly 1 min without touching the skin surface. Finally the solvent was withdrawn using a Pasteur pipette. The edge of the sampling site was marked with a marker pen and covered with a light gauze which was kept away from the skin by a polystyrene ring. After 1 h and 2 h the procedure was repeated. The samples were then evaporated under a stream of nitrogen and the residue stored at −18°C. Samples were taken at the same time of day on each of the three occasions. All scrubs were carried out by the same person to reduce sampling error.

### *Microbiology*

Tenfold dilutions of the samples in scrubbing solution were prepared and viable counts were obtained using the method of Miles, Misra & Irwin (1938). A standard medium TSYT agar was used for aerobic and anaerobic cultures (3% Tryptone Soya Broth, Oxoid, 1% yeast extract, 0·5% Tween 80, 1·2% agar).

Aerobic cultures were incubated for 24 h at 37°C, the colonies were counted, and each colony type was subcultured and Gram stained. *S. aureus* was identified by coagulase and DNAase test by standard methods (Cowan & Steel, 1974). No distinction was made between coagulase negative staphylococci and micrococci.

Anaerobic cultures were incubated in anaerobe jars containing a cold catalyst in an atmosphere of 95% $H_2$ 5% $CO_2$ at 37°C for 7 days. Propionibacteria were identified by colony morphology, Gram stain, and catalase test and differentiated according to the scheme of Marples & McGinley (1974) by means of nitrate reduction, indol production, DNAase production (standard methods, Cowan & Steel, 1974), gelatin liquefaction and casein hydrolysis (McGinley, Webster & Leyden, 1978).

Microorganisms other than aerobic cocci and Propionibacteria were only found occasionally and were therefore omitted from the analyses.

### *Lipid assay*

Qualitative and quantitative analysis of lipid samples was carried out by thin layer chromatography (TLC) using a modification of the method described by Greene *et al.* (1970). T.l.c. was performed on ready-made silica gel plates (Schleicher+Schüll); the solvent system consisted of hexane (to 19 cm), hexane/diethylether 90:10 (to 19 cm) and hexane/diethylether/acetic acid glacial 70:30:1 (to 12 cm) which produced more consistent results than the solvent system used by Greene *et al.* (1970). The developed plates were charred by spraying with 50% sulphuric acid in methanol and heating up to 220°C. The lanes were scanned on a densitometer (Vitatron TLD 100) and the percentages of the lipid components calculated from the integrator counts; the total amount of lipid was computed using the internal standard. A standard mixture of neutral lipids (SIGMA) served as a day-to-day quality control. Correction factors proposed by Downing (1968) were not applied as all tests were carried out under the same conditions and the variations of the SIGMA standards were relatively low.

The following fractions could be separated: cholesterol (including diglycerides), free fatty acids, triglycerides, methyl ester (internal standard), wax esters (including cholesterol esters), squalene and hydrocarbons. The latter were not included in the calculations. Emulsifying ointment and aqueous cream appeared in the hydrocarbon fraction and in a separate band between cholesterol and fatty acids and thus, if present, did not influence the results.

## RESULTS

### *Microbiology*

The total count of aerobic cocci from the interscapular region of the patients remained unchanged but there was a significant increase of aerobic cocci on the forearm during the first phase of photochemotherapy (Table 1), mainly due to a significant rise in the number of *Staphylococcus aureus* (Table 2). The density of *S. aureus* decreased to initial levels at the end of the treatment. Only one patient showed a marked increase of *S. aureus* throughout therapy; this patient (J.M.) developed boils on his legs from which *S. aureus* was isolated. The incidence and density of *S. aureus* on the back of the patients was relatively low except in one case.

Levels of propionibacteria on the forearm were, as expected, relatively low and did not show considerable changes. Viable counts of propionibacteria on the back were higher and a clear distinction in low-count and high-count individuals could be seen (Table 3). The high-count individuals showed a slight decrease of propionibacteria levels at the end of the PUVA treatment, which was not statistically significant. Logarithmic counts of propionibacteria from the inter-scapula region were significantly correlated with skin lipid production (replacement sum) before PUVA ($r = 0{\cdot}731$; $P<0{\cdot}01$) and during the first period of treatment ($r = 0{\cdot}690$; $P<0{\cdot}02$) but not at the end of the treatment ($r = 0{\cdot}221$).

Fifty-nine of eighty isolates of propionibacteria tested had biochemical reactions characteristic of *P. acnes* (McGinley *et al.*, 1978); six isolates were identified as *P. granulosum* and seven as *P. avidum*. Eight isolates did not fit into this scheme and are subject to further investigation. Except for one patient (J.B.), who had large numbers of these unclassifiable organisms on his back in all three samples, *P. acnes* was by far the most prevalent microorganism. *P. granulosum*, if present, never exceeded 10% of the total counts of propionibacteria. *P. avidum* was occasionally found on the arm in very low numbers, probably as a transient rather than a resident member of the flora. The incidence and numbers of *P. granulosum* and *P. avidum* did not show any correlation to the treatment.

### *Skin lipids*

In the course of the therapy there was a marked increase in the amount of lipid recovered from both

TABLE 1. Logarithmic counts of total aerobic cocci during PUVA treatment of psoriasis

| | Forearm (log cfu/cm$^2$) | | | Back (log cfu/cm$^2$) | | |
|---|---|---|---|---|---|---|
| Patient | Before | During | After | Before | During | After |
| K.B. | — | 2·88 | 1·70 | — | 1·11 | 1·89 |
| F.G. | 2·00 | 2·12 | 1·61 | 2·26 | 2·23 | 1·48 |
| C.W. | 4·02 | 4·42 | 4·44 | 5·36 | 5·54 | 5·10 |
| P.G. | 3·67 | 3·82 | 3·23 | 2·99 | 2·45 | 3·00 |
| J.M. | 3·10 | 3·88 | 4·42 | 3·86 | 3·18 | 4·29 |
| T.G. | 2·64 | 2·56 | 2·43 | 1·86 | 2·66 | 1·82 |
| H.H. | 3·87 | 4·22 | 3·51 | 2·56 | 2·04 | 3·04 |
| D.M. | 2·62 | 2·95 | 2·53 | 0·90 | 1·56 | 0·90 |
| J.B. | 4·17 | 4·52 | 3·78 | 5·23 | 5·20 | 4·56 |
| D.O. | — | 1·96 | 1·56 | 2·30 | 2·29 | 1·30 |
| Mean | 2·61 | 3·33 | 2·92 | 2·73 | 2·83 | 2·74 |
| S.d. | 1·46 | 0·91 | 1·06 | 1·63 | 1·38 | 1·42 |
| | $P < 0·05$ | | | | | |

— = no organisms isolated.

TABLE 2. Logarithmic counts of *S. aureus* during PUVA treatment of psoriasis

| | Forearm (log cfu/cm$^2$) | | | Back (log cfu/cm$^2$) | | |
|---|---|---|---|---|---|---|
| Patient | Before | During | After | Before | During | After |
| K.B. | — | — | — | — | — | — |
| F.G. | — | — | — | 1·78 | — | — |
| C.W. | 3·29 | 4·00 | — | 4·02 | 4·78 | 4·72 |
| P.G. | 2·47 | 2·92 | 2·20 | 2·65 | — | — |
| J.M. | 2·81 | 3·88 | 4·42* | 2·57 | — | — |
| T.G. | — | — | — | — | — | 1·53 |
| H.H. | 3·38 | 4·21 | — | — | — | 2·64 |
| D.M. | 1·78 | 2·17 | 2·48 | 0·30 | — | — |
| J.B. | — | 4·35 | 3·34 | — | — | — |
| D.O. | — | 1·81 | — | 2·65 | | |
| Mean† | 1·92 | 3·33 | 1·77 | | | |
| S.d. | 1·32 | 0·96 | 1·67 | | | |
| | $P < 0·05$ | | | | | |

* This patient developed folliculitis on his legs.

† n = 7; K.B., F.G. and T.G. are omitted.

— = no organisms isolated.

TABLE 3. Logarithmic counts of propionibacteria during PUVA treatment of psoriasis

| | Forearm (log cfu/cm$^2$) | | | Back (log cfu/cm$^2$) | | |
|---|---|---|---|---|---|---|
| Patient | Before | During | After | Before | During | After |
| K.B. | 2·15 | 0·80 | 1·79 | 5·82 | 5·08 | 2·00 |
| F.G. | 1·65 | 1·26 | 1·32 | 1·73 | 1·56 | 1·59 |
| C.W. | — | 2·88 | — | — | — | 1·41 |
| P.G. | — | 1·26 | 2·00 | 5·69 | 5·00 | 5·72 |
| J.M. | 1·73 | 0·90 | — | 5·34 | * | 5·17 |
| T.G. | — | — | — | — | 0·90 | — |
| H.H. | 0·90 | 3·16 | 2·25 | 4·88 | 5·62 | 4·31 |
| D.M. | 1·60 | — | 0·78 | — | — | — |
| J.B. | 1·51 | 1·20 | 1·53 | 4·90 | 6·09 | 4·41 |
| D.O. | 2·00 | 2·16 | 2·42 | 3·81 | 4·38 | 1·41 |

* Culture lost due to failure of anaerobic conditions.
— = no organisms isolated.

sampling sites (Fig. 1). This rise was significant for the samples obtained from unprepared and unprotected areas (casual level) as well as for the replacement sum (arithmetical mean of the samples after 1 and 2 h, modified from Herrmann & Prose (1951)) on the arm. The details of skin lipid composition are shown in Tables 4 and 5. While the percentages of cholesterol, wax and cholesterol-esters and squalene remained more or less unchanged, there was an increase of free fatty acids at the expense

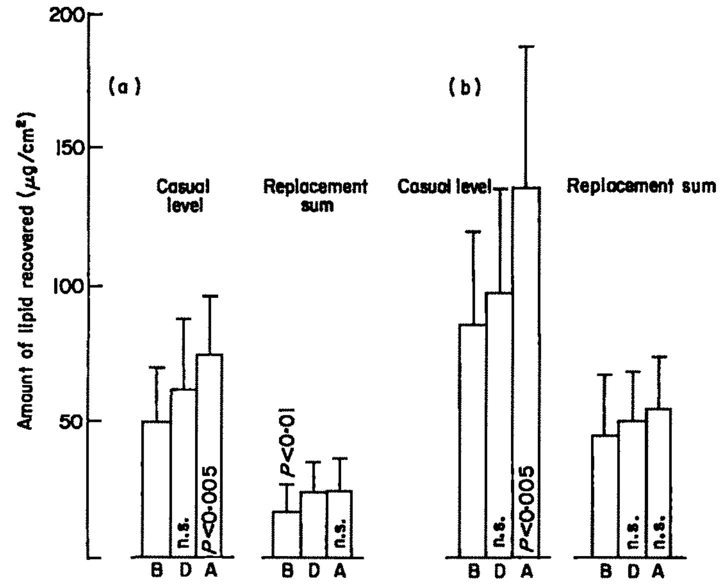

FIGURE 1. Skin surface lipids in ten patients receiving PUVA treatment. (a) Arm; (b) back. B = before treatment; D = during; A = after.

TABLE 4. Skin lipid composition of ten patients receiving PUVA treatment (mean ± s.d.)—casual lipids

| | Forearm | | | Back | | |
|---|---|---|---|---|---|---|
| | Before | During | After | Before | During | After |
| Total lipid ($\mu g/cm^2$) | 51·1 ± 19·7 | 62·1 ± 26·0 | 75·3 ± 22·6 | 86·2 ± 34·4 | 96·7 ± 31·6 | 135·8 ± 50·6 |
| Cholesterol (%) | 10·1 ± 6·5 | 12·7 ± 5·7 | 11·7 ± 6·5 | 10·1 ± 3·3 | 10·4 ± 4·3 | 8·4 ± 4·0 |
| Fatty acids (%) | 29·6 ± 9·8 | 33·5 ± 9·9 | 27·9 ± 9·1 | 17·8 ± 7·2 | 25·0 ± 7·5 | 22·0 ± 9·7 |
| Triglycerides (%) | 32·3 ± 11·6 | 24·9 ± 5·7 | 29·7 ± 6·5 | 35·0 ± 8·4 | 26·3 ± 5·8 | 29·6 ± 5·5 |
| Esters (%) | 18·6 ± 5.2 | 19·5 ± 6·4 | 21·2 ± 3·5 | 23·9 ± 5·7 | 23·4 ± 6·1 | 26·0 ± 6·4 |
| Sqalene (%) | 9·6 ± 6·0 | 9·5 ± 8·4 | 9·5 ± 6·9 | 13·1 ± 4·3 | 13·8 ± 5·4 | 14·3 ± 6·5 |

TABLE 5. Skin lipid composition of ten patients receiving PUVA treatment (mean ± s.d.)—replacement lipids

| | Forearm | | | Back | | |
|---|---|---|---|---|---|---|
| | Before | During | After | Before | During | After |
| Total lipid ($\mu g/cm^2$) | 17·6 ± 9·9 | 24·5 ± 11·5 | 25·0 ± 13·9 | 45·1 ± 23·2 | 52·2 ± 17·2 | 56·1 ± 18·3 |
| Cholesterol (%) | 13·7 ± 6·7 | 16·8 ± 6·0 | 13·6 ± 6·1 | 9·6 ± 4·9 | 11·3 ± 3·1 | 9·8 ± 4·5 |
| Fatty acids (%) | 21·5 ± 7·2 | 24·6 ± 3·8 | 28·6 ± 12·3 | 16·2 ± 7·3 | 20·6 ± 3·4 | 21·5 ± 12·3 |
| Triglycerides (%) | 28·6 ± 10·6 | 29·0 ± 5·6 | 29·8 ± 7·8 | 37·4 ± 12·7 | 33·7 ± 5·7 | 32·6 ± 7·4 |
| Esters (%) | 28·4 ± 6·6 | 21·4 ± 3·6 | 21·2 ± 5·3 | 25·7 ± 7·0 | 22·2 ± 3·8 | 23·5 ± 4·8 |
| Sqalene (%) | 6·8 ± 3·4 | 6·7 ± 5·0 | 6·8 ± 6·2 | 10·6 ± 4·0 | 11·5 ± 5·1 | 12·7 ± 6·5 |

of triglycerides (except for the replacement lipids of the forearm). These changes, however, were not statistically significant.

## DISCUSSION

Amongst the results of this study, three findings seem to be of particular interest: the increase of skin lipid excretion during PUVA therapy, the relative decrease of the density of propionibacteria, and the transient rise of aerobic cocci on the arm. This increase, mainly of *S. aureus*, seems not to be directly related to the PUVA treatment. It is rather suggested that these organisms originate from the psoriatic lesions which are known to have a high incidence and density of *S. aureus* (Marples, Heaton & Kligman, 1973). The application of creams and ointments during the course of PUVA treatment, which is supposed to aid in removing scales and preventing excessive dryness (Lynch & Roenig, 1977), could result in spread of the bacteria from lesions to surrounding uninvolved skin; the survival of these organisms could even be enhanced by a possible occluding and/or nutrient effect of the creams or ointments. This presumption is supported by the fact that *S. aureus* was found only occasionally and in rather low numbers in the interscapular region, which, for obvious reasons, is not so easily accessible for ointment treatment. Furthermore *S. aureus* counts returned to pre-treatment levels at the end of PUVA therapy when the lesions had cleared. In this context it seems important to mention that one patient with steadily rising *S. aureus* counts developed follicular staphylococcal pustules on his legs which required antibiotic treatment.

The results of the lipid investigation do not support the common belief that UV irradiation leads to a reduction in sebum production. The views about the benefit of ultraviolet treatment for acne vulgaris are still controversal. Recently Mills & Kligman (1978) investigated the effect of ultraviolet phototherapy and photochemotherapy on acne. They found only minimal clinical improvement under UV-B treatment and no effect at all of UV-A alone or in combination with 8-MOP. Unfortunately this trial did not include skin lipid investigations. Gloor & Karenfeld (1977), using a similar solvent extraction method as in the present paper, found a significant increase in the amount of skin lipids in volunteers who had been receiving UV treatment for several weeks. The light source used by these workers was a Höhensonne (Q 600T Original Hanau) emitting UV-A and UV-B light. A different light source (Osram HBO 200) producing only a small peak in the UV-B range (313 nm) and the vast majority in the UV-A spectrum (peak at 366 nm) was employed by Dachs & Plewig (1977) for their animal experiments. Using auto-radiography and planimetrical methods they were able to show a marked increase in size and mitotic index of the sebaceous ear glands of the Syrian hamster after UV irradiation, suggesting a stimulation of sebum production by UV-A light. This investigation however did not include experiments with 8-MOP and UV-A light.

At present it cannot be determined whether the increase in skin lipid production in psoriatics receiving PUVA treatment is due to the ultraviolet irradiation alone or to a combined effect of UV-A and 8-MOP. A control group of psoriatic patients receiving only UV-A irradiation was not available and the patients who were treated with dithranol and sun lamp could not be regarded as a control group for skin lipid and skin bacteria investigations, as this treatment includes too many variables (Rogers *et al.*, 1979).

The assumption that PUVA, which is known to reduce the turnover of fast proliferating cells, would also inhibit the proliferation of sebocytes could not be verified in this investigation. The increase of extractable skin lipid in our patients indicates that the sensation of 'dryness' experienced by many patients under PUVA treatment is not due to a lack of emulsifying substances on the skin surface. These findings, and the possible influence of creams and ointments on skin bacteria described above, raise the question whether patients should always receive ointments and creams for the whole duration of PUVA treatment. Perhaps they should be employed only in the first phase of treatment, to help remove scales and later only if patients have the subjective feeling of tenderness or dryness of their skin.

The influence of photochemotherapy on the quantitative bacteriology of the skin seems to be relatively small at the first glance, if one considers the massive effects of UV-A light in combination with 8-MOP on various microorganisms *in vitro*. Oginski *et al.* (1959) demonstrated the lethal effect of psoralens and long wave ultraviolet light on *S. aureus* and *Escherichia coli*. Fujita & Suzuki (1977) found, in addition to this lethal effect, induction of phage and mutations in *E. coli* sensitized with 8-MOP; this was dependent on the wavelength of UV irradiation. The most effective wavelength was between 320 and 340 nm, a range which is part of the spectrum of PUVA light sources. Scherwitz, Rassner & Martin (1978) found severe ultrastructural cell damage in *Candida albicans*. Oberste-Lehn & Plempel (1977) investigated the bacteriostatic effect of 8-MOP and blacklight on several microorganisms using an 8-MOP disk diffusion technique. They demonstrated an inhibition of growth of *S. aureus*, *S. epidermidis*, Gram negative bacteria, yeasts and dermatophytes but were able to reculture some of these organisms from the zones of inhibition. They also reported a fungistatic effect of PUVA in experimental trichophyton infection of guinea-pigs. *In vivo* investigations on the effects of PUVA on the bacterial flora of human skin have, to our knowledge, not yet been carried out. The resident bacterial flora of the patients we investigated seemed to be relatively stable. A substantial reduction of the microbial population, anticipated from *in vitro* studies, could not be detected. Nevertheless a direct effect of PUVA on the microorganisms cannot be ruled out; the reduction of propionibacterium counts on the back of the patients, although not statistically significant, should not be underestimated. While the population of *P. acnes* at the beginning of the treatment was dependent on the amount of sebum, this correlation was lost at the end of the treatment. The discrepancy between increasing amounts of skin lipid and reduced density of propionibacteria indicates a possible influence of the therapy on the bacterial ecology. Whether this represents a direct effect of PUVA on the bacteria or a secondary action due to a change of host factors cannot be decided at present.

Although no drastic changes in the skin flora were found in this investigation, the importance of possible effects of PUVA on the microbial ecology of human skin is not diminished, as qualitative changes, such as an influence on the genetics of the microorganisms, would not be detected by our methods. Genetic changes in skin organisms may have clinical importance (Noble & Naidoo, 1978). Both acquisition and transfer of antibiotic resistance plasmids occur in bacteria on the skin. Very high level resistance to gentamicin was acquired by the *S. aureus* strain on the skin of a patient who in addition to topical antibiotic treatment received PUVA therapy (Naidoo & Noble, 1978).

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***Pharmacology and Treatment***

# Systemic treatment of psoriasis with an oral retinoic acid derivative (Ro 10-9359)

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## SUMMARY

Ninety-seven patients with severe psoriasis took part in a 1-year study to evaluate the effect of a new oral synthetic retinoid (Ro 10-9359). The trial was performed in a double-blind cross-over fashion. The treatment started with either 100 mg daily of Ro 10-9359 or placebo and the maintenance dose was in most cases 50 mg. Follow-up examinations were performed monthly and the parameters erythema, desquamation, infiltration and extent of the lesions were followed. Throughout the study there was a significant to highly significant preference for Ro 10-9359 shown by all parameters. More patients were in complete remission after Ro 10-9359 periods than after placebo periods. The side-effects of Ro 10-9359 on uninvolved skin and mucous membranes seemed to be largely dose-dependent. Twenty-three patients interrupted the study, four of them because of side-effects, mainly alopecia. Laboratory examinations revealed no aberrations which could be attributed to the therapy. One patient developed hepatitis during a placebo period.

The retinoid Ro 10-9359 is an analogue of vitamin A acid or retinoic acid. It is very similar to retinoic acid with the same tetraene side chain which causes the characteristic photosensitivity (Fig. 1). The two structures differ in the ring moiety and the end group. Retinoic acid has a non-aromatic, partly unsaturated ring and a carboxy group, while Ro 10-9359 has an aromatic ring and an ester group.

Different retinoids have earlier been reported to show moderate antipsoriatic activity (Schumacher & Stüttgen, 1971; Orfanos *et al.*, 1972; Runne, Orfanos & Gartmann, 1973). During recent years several authors have reported a good response to oral administration of Ro 10-9359 in severe cases of psoriasis (Ott & Bollag, 1975; Orfanos & Runne, 1976; Schimpf, 1976; Dahl, Møllenbach & Reymann, 1977; Ott, 1977; Fredriksson & Pettersson, 1978; Fritsch *et al.*, 1978). So far, however, all studies on this subject have been open and uncontrolled. The present study was carried out as a double-blind cross-over trial in cases with severe psoriasis. Ro 10-9359 was compared with placebo and the study extended for 12 months in order to eliminate spontaneous seasonal variations in the clinical course of the disease.

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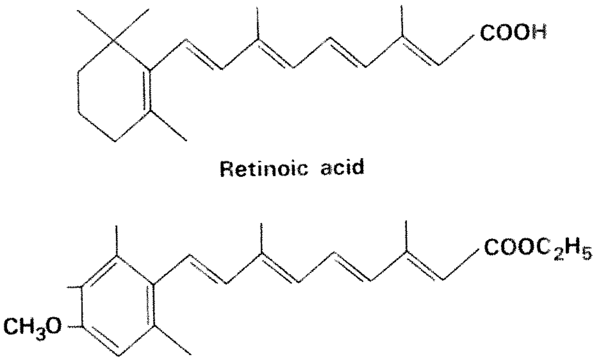

FIGURE 1. Chemical structures of retinoic acid and retinoid Ro 10-9359.

## MATERIAL AND METHODS

Ninety-seven adult members of the Psoriasis Association in Finland volunteered for the study. They all had severe psoriasis of long duration (Table 1). The series was divided into two groups, group A (forty-eight patients) and group B (forty-nine patients).

The trial was performed in a double-blind cross-over fashion during a period of 12 months. Ro 10-9359 was compared with placebo. The treatment started with either Ro 10-9359 or placebo and after 3, 6 and 9 months a cross-over to the comparative preparation was made (Fig. 2). Thus, each patient was treated with both Ro 10-9359 and placebo during two different periods of 3 months. Concomitant local therapy including emollient ointments or local corticosteroid preparations was allowed if the patient found it necessary. The patients were allocated randomly to the treatment schedules.

Bottles containing 300 tablets of 25 mg of Ro 10-9359 or placebo were provided for 3 months at a time to each patient. The initial dose was 100 mg/day for 2 weeks and was then decreased to 50 mg/day for the rest of the first period. In some cases the dosage had to be temporarily decreased to 25 mg/day because of side-effects (Table 2). After each cross-over the dosage was adjusted to the same level as during the last month of the preceding period, i.e. in the majority of cases two tablets or 50 mg/day.

The patients were seen monthly throughout the study. Judgement of the severity of the disease at

TABLE 1. Clinical data of patients of groups A and B

| | Group A | Group B |
|---|---|---|
| Total number of patients | 48 | 49 |
| Females | 31 | 20 |
| Males | 17 | 29 |
| Age (years) Mean ± s.e. | 43·3 ± 2·2 | 42·0 ± 2·0 |
| Weight (kg) Mean ± s.e. | 66·4 ± 1·6 | 74·4 ± 2·1 |
| Duration of disease (years) | | |
| Mean ± s.e. | 17·5 ± 1·8 | 17·9 ± 1·7 |
| Type of psoriasis: | | |
| Psoriasis vulgaris | 46 | 49 |
| Erythrodermia | 1 | |
| Generalized pustular psorasis | 1 | |

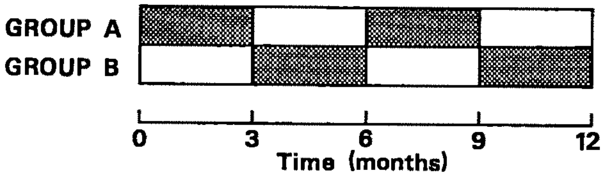

FIGURE 2. Design of the trial. (Stipple) Ro 10-9359; (open boxes) placebo.

TABLE 2. Reduced dosage of Ro 10-9359 (50 mg/25 mg)

| | 0 | First period | Second period | Third period | Fourth period |
|---|---|---|---|---|---|
| Group A | | | | | |
| Ro/P/Ro/P | — | 17 | 14 | 19 | 18 |
| Total | 48 | 48 | 41 | 40 | 39 |
| Group B | | | | | |
| P/Ro/P/Ro | — | 2 | 16 | 14 | 20 |
| Total | 49 | 47 | 42 | 36 | 35 |

P = placebo.
Ro = Ro 10-9359.

the start of therapy as well as at each visit was made on the basis of the following parameters: erythema, desquamation, and infiltration. The severity of these parameters was graded from 0–4. Extent of the lesions was expressed as approximate percentage of involved skin.

At each follow-up visit effects on the healthy skin, mucous membranes, hair and nails were recorded. Further symptoms like burning, itching, sweating and thirst were also recorded. Before the trial began and thereafter every third month the following laboratory parameters were examined: erythrocyte sedimentation rate, red and white blood cell counts (including differential count), platelet count, serum transaminases, alkaline phosphatase, serum creatinine and urinalysis.

## RESULTS

Altogether seventy-four of the ninety-seven patients completed the whole study. Thirteen of group A and ten of group B interrupted the therapy for different reasons which will be presented in detail later. Interruptions occurred in eleven cases during or after active treatment and in twelve cases during or after placebo periods.

Before the treatment started there were no statistically significant differences between the severity of erythema, desquamation or infiltration of groups A and B. In Table 3 the two groups have been compared with each other after each period of 3 months of treatment. After the two first periods there was a highly significant preference for Ro 10-9359 for all three parameters. After the third period the preference for the active treatment was less significant for erythema and infiltration ($P<0{\cdot}01$) but was still highly significant for desquamation. After the fourth period the preference for the active treatment was still significant for all three parameters ($P<0{\cdot}01$).

The influence of the treatment on the extent of psoriasis is presented in Figs 3 and 4. In Fig. 3, group B which started with placebo is illustrated. During the first 3 months of placebo treatment the extent stayed approximately the same and thereafter dropped significantly during the second 3

TABLE 3. Significance of the effect of Ro 10-9359 on erythema, desquamation and infiltration of psoriatic lesions as compared with placebo; $\chi^2$-test

| Results | Erythema | | Desquamation | | Infiltration | |
|---|---|---|---|---|---|---|
| After first period | Placebo | Ro 10-9359 | Placebo | Ro 10-9359 | Placebo | Ro 10-9359 |
| Amelioration + complete remission | 21 | 40‡ | 23 | 42‡ | 22 | 43‡ |
| No change + worsening | 26 | 8 | 24 | 6 | 25 | 5 |
| After second period | Ro 10-9359 | Placebo | Ro 10-9539 | Placebo | Ro 10-9359 | Placebo |
| Amelioration + complete remission | 36 | 10‡ | 37 | 12‡ | 36 | 10‡ |
| No change + worsening | 6 | 31 | 5 | 29 | 6 | 31 |
| After third period | Placebo | Ro 10-9359 | Placebo | Ro 10-9359 | Ro 10-9359 | Placebo |
| Amelioration + complete remission | 4 | 18† | 3 | 28‡ | 4 | 20‡ |
| No change + worsening | 32 | 22 | 33 | 12 | 32 | 20 |
| After fourth period | Ro 19-9359 | Placebo | Ro 10-9359 | Placebo | Placebo | Ro 10-9359 |
| Amelioration + complete remission | 20 | 9† | 22 | 11† | 22 | 11† |
| No change + worsening | 15 | 30 | 13 | 28 | 13 | 28 |

$\chi^2$-test with Yate's correction.
* = 0·05; † = 0·01; ‡ = 0·001.

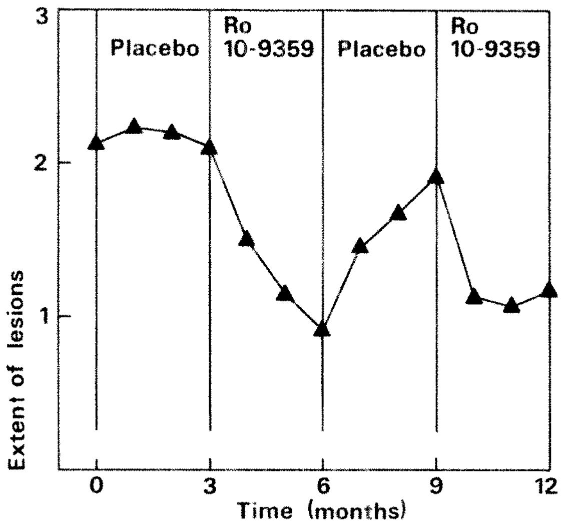

FIGURE 3. Changes in the extent of psoriasis in the patients of group B. (1) 1–10% involved; (2) 11–30% involved; (3) 31–50% involved.

months of active treatment. During the following placebo period the extent gradually grew again to almost the same level as before the trial but again showed a marked decrease during the fourth period. Figure 4 illustrates the changes of extent in group A which started with active treatment. The trend of the changes is the same as in group B but the differences between the Ro 10-9359 and placebo periods are not so marked.

The numbers of patients in complete remission after the different periods are shown in Table 4. Definitely more patients were completely free of symptoms of psoriasis after periods on Ro 10-9359 than after placebo periods.

The use of local treatment was recorded at each visit. All patients used some bland emollient oint-

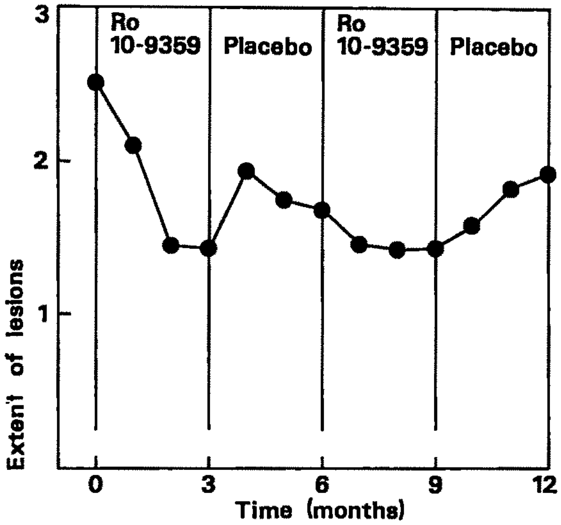

FIGURE 4. Changes in the extent of psoriasis in the patients of group A. (1) 1–10% involved; (2) 11–30% involved; (3) 31–50% involved.

TABLE 4. Number of patients in complete remission after the different periods of treatment

| | Group A | Group B |
|---|---|---|
| First period | Ro 10-9359<br>8/48 (17%) | Placebo<br>3/47 (6%) |
| Second period | Placebo<br>5/41 (12%) | Ro 10-9359<br>19/42 (45%) |
| Third period | Ro 10-9359<br>12/40 (30%) | Placebo<br>3/36 (8%) |
| Fourth period | Placebo<br>3/39 (8%) | Ro 10-9359<br>7/35 (20%) |

ment throughout the study and temporary use of some local corticosteroid preparation was frequent. Such therapy was more frequently used during periods of placebo treatment.

The effects of the treatment on uninvolved skin are shown separately for groups A and B (Table 5). The reason for this is that the groups do not match as to sex and body weight as group B included more men and the mean body weight was consequently considerably higher in this group (Table 1). In addition, the patients in group A received an initial dose of 100 mg/day of Ro 10-9359 for the first 2 weeks while the patients in group B had a dose of 50 mg/day throughout the whole first period. In Table 5 the side-effects after the first and last month of active treatment are shown. When comparing groups A and B there was a definite difference in the effects on uninvolved skin after the first month of active treatment. The most frequent side-effects in both groups were erythema and scaling which were much more prominent in group A. Thinning, rhagades and ulceration of the skin occurred only in group A in which also itching was a much more prominent feature than in group B. Table 5 also shows the effects on uninvolved skin in both groups after the last month of active treatment. Side-

TABLE 5. Side-effects of Ro 10-9359 after first and last month of active treatment

| | Side-effect present (%) | | | |
|---|---|---|---|---|
| | After first month | | After last month | |
| | Group A | Group B | Group A | Group B |
| Uninvolved skin | | | | |
| Erythema | 65 | 25 | 17 | 8 |
| Desquamation | 50 | 23 | 14 | 5 |
| Palmo-plantar desquamation | 60 | 18 | 8 | 0 |
| Thinning of the skin | 4 | 0 | 0 | 0 |
| Rhagades and ulceration | 6 | 0 | 0 | 3 |
| Burning | 2 | 4 | 0 | 0 |
| Itching | 23 | 4 | 0 | 8 |
| Mucous membranes | | | | |
| Dryness—lips | 77 | 47 | 42 | 21 |
| Dryness—nasal mucosa | 56 | 25 | 33 | 10 |
| Dryness—oral mucosa | 48 | 23 | 14 | 3 |
| Conjunctivitis | 10 | 0 | 0 | 0 |
| Mucosal erosions | 10 | 2 | 0 | 0 |
| Other side-effects | | | | |
| Alopecia | 4 | 2 | 6 | 8 |
| Splitting of nails | 0 | 0 | 3 | 3 |
| Paronychia | 8 | 0 | 0 | 0 |
| Thirst | 4 | 0 | 0 | 0 |
| Stomach pain | 2 | 0 | 0 | 0 |
| Sweating | 2 | 4 | 0 | 3 |

effects were still more frequent in group A but the differences were only slight and uncommon in both groups.

The most frequent side-effects of mucous membranes were dryness of lips and nasal and oral mucosae (Table 5). These effects were more frequent in group A throughout the study. Mucosal erosions and conjunctivitis occurred only after the first month and with one exception only in group A.

Other side-effects were infrequent (Table 5). Paronychia, thirst and abdominal pain occurred only in group A and were totally absent at the end of the last month of active treatment. A few patients complained of sweating during the whole study. The greatest frequency of alopecia was seen during the second and third month of active treatment: 21% of the patients in both groups had significant hair loss during these months. There was also a considerable overlap of hair loss in the following placebo period in both groups, which was not seen with other side-effects.

The laboratory examinations revealed no aberrations which could be attributed to the therapy. Transient fluctuations in the serum transaminases occurred but were obviously due to the use of alcohol. With one exception the alkaline phosphatase values remained within normal limits during the whole study. This patient is described in detail below. Minor changes in the red and white cell counts occurred, but were attributable rather to the disease itself than to the therapy. The therapy did not affect the serum creatinine level or cause abnormal findings in the urine.

Altogether four patients interrupted the treatment because of side-effects, in three cases because of alopecia and in one because of severe paronychia. All four belonged to group A and the interruption occurred after the first period. A follow-up examination 3 months after the interruption showed that the side-effects had been reversible in all four cases. Four patients interrupted because of an exacerbation of the disease during a placebo period. They were all changed to PUVA therapy to which they responded well. Four patients were excluded from the trial because of alcoholism, two of these during the first period. Six patients interrupted because of some concomitant disease. In four cases this was a circulatory disease (one of them died of myocardial infarction), in one case various neurotic symptoms and in one hepatitis. This last patient will be described in more detail. Five patients interrupted for other reasons.

A 48-year-old woman joined the trial (group B) in September 1976. She had widespread psoriasis vulgaris of long duration without concomitant disease. She was a moderate user of alcohol. She started the first active treatment period in December 1976 and finished it in February 1977. At the end of this period all her laboratory values, including serum transaminases and alkaline phosphatase, were within normal limits. In April 1977, during her second placebo period, she had an acute gastroenteritis with fever. Four weeks later she was found to be icteric with elevated serum transaminase and alkaline phosphatase values. An examination at a hospital for internal medicine resulted in a diagnosis of probable viral hepatitis. The histopathological picture was that of chronic aggressive hepatitis. She recovered completely after oral corticosteroid treatment.

## DISCUSSION

The mechanism by which retinoic acid and its derivatives act on the proliferation and differentiation of human epithelial tissue is still entirely unknown. Some new retinoids like Ro 10-9359 seem to possess greater specificity with less toxicity. The tissue changes in the psoriatic plaque after systemic administration of Ro 10-9359 were described by Orfanos & Runne (1978). In addition to enhanced inflammation and extracellular accumulation of mucus-like material they observed signs of normalization of cell differentiation with reappearance of keratohyalin.

The results of the present long-term, controlled study established that oral Ro 10-9359 is a potent antipsoriatic agent. Throughout the whole study the effect of Ro 10-9359 was significantly better than that of placebo. The difference between Ro 10-9359 and placebo was more marked during the two first periods of group B which started with placebo, as shown for example by the changes in the extent of the lesions. This suggests that in group A there was an overlap of the retinoid effect in the later placebo periods. The same was obviously true also for the second half year of the study of group B. This indicates that Ro 10-9359 might have a prolonged effect on the psoriatic lesions after the treatment has been completed. The overall results of the present trial agree well with the results presented at a recent workshop (Fredriksson, 1978; Goerz & Orfanos, 1978; Dominguez Soto & Hojyo-Tomoko, 1978; Viglioglia & Barclay, 1978).

Concomitant local therapy with corticosteroids probably affected the results very little and only reduced the differences between the periods of active treatment and placebo. The use of local therapy if found necessary was allowed because of the long-term nature of the trial. The side-effects of Ro 10-9359 on uninvolved skin and mucous membranes seemed to be largely dose-dependent. The frequency of such side-effects was highest in group A after the high initial dose but comparatively low in group B throughout the study and in group A during the second part of the trial. The frequency of these side-effects was in all phases of the trial higher in group A, reflecting the difference in body weight between the two groups. This indicates that Ro 10-9359 should be administered according to body weight. From the present study a dose of 1 mg or less per kg body weight per day should be the correct amount in most cases.

Side-effects like paronychia and thirst seemed also to be dose-dependent. Alopecia on the other hand developed equally in both groups and later than the other side-effects. Hair loss was the main side-effect to cause interruption of the treatment in the present series. Overall the side-effects in the present study were frequent but mainly mild and reversible in all cases.

Blood status and renal function did not seem to be impaired by the treatment, neither were there signs of liver damage except in one case. It is impossible to state definitely whether the hepatitis in this case was related to therapy or not. Fredriksson (1978) reported two cases of possible Ro 10-9359 induced liver damage including bilirubinaemia and elevated transaminases. In his cases too liver biopsy revealed a picture of chronic aggressive hepatitis. The observations of Fredriksson (1978) and the present case indicate that serum transaminases and alkaline phosphatase should be followed monthly during systemic use of Ro 10-9359.

Although the mechanism of action of systematically administered retinoid on psoriasis is still unknown the results of the present study further stress the role of Ro 10-9359 as a new potential antipsoriatic agent.

## ACKNOWLEDGMENTS

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# Combined treatment of psoriasis with a new aromatic retinoid (Tigason) in low dosage orally and triamcinolone acetonide cream topically: a double-blind trial

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## SUMMARY

In a multicentre double-blind trial the effect of three therapy regimens was studied for 6 weeks in ninety psoriasis patients:

(1) aromatic retinoid (Ro 10-9359) orally (0·50–0·66 mg/kg body weight) and placebo cream topically;

(2) aromatic retinoid (Ro 10-9359) (same dosage) with 0·1% triamcinolone acetonide and 5% salicylic acid in lanette wax cream;

(3) placebo capsules with 0·1% triamcinolone acetonide and 5% salicylic acid in lanette wax cream.

Regimen 1 had virtually no effect and regimen 2 gave better results than regimen 3 for almost all parameters, although statistical significance was reached for only some of them.

The 6 week double-blind period was followed by an open study in which all patients were treated according to regimen 2. The clinical result could be maintained up to the end of the study (18 weeks), when more than 60% of the patients showed good to excellent (80–100%) improvement. Most of the patients had dry lips, but generally without clinical symptoms of a cheilitis. The other well-known side-effects of retinoid were mild and relatively rare.

It is concluded that the combination of the aromatic retinoid (Ro 10-9359) given in low dosage orally with corticosteroids topically is as effective as therapy with the retinoid in high dosage alone, but with markedly less side-effects.

Retinoids have a well-known effect on the differentiation of epithelia, and have therefore been used for many years, both orally and topically, in the treatment of several dermatoses. Orally administered

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retinoids, in particular vitamin A acid, have been found effective, but serious side-effects, especially dry mucous membranes, headache, hair loss, and liver damage, have limited the therapeutic use of this drug. In 1975, Ott & Bollag reported very good results with the new aromatic retinoid (Ro 10-9359), Tigason, in the treatment of psoriasis. Since then, increasing numbers of publications on the clinical results obtained with this drug have appeared (e.g. *Dermatologica*, **157**, Suppl. I, 1978). Although in comparable dosage Tigason is tolerated considerably better than vitamin A acid, most authors report a notable list of side-effects at currently used dosages, the most important being dryness of lips, mouth, and nose, sometimes with rhagades, liver function disturbances, and, after several weeks of administration, hair loss.

The usual dosage used for the initial treatment with the aromatic retinoid is 1 mg/kg body weight. After 4–8 weeks the dose is often decreased to a maintenance level of 0·5–0·75 mg/kg body weight. In a pilot study we used this schedule and found that although the clinical results were good, the side-effects were often unacceptable for the patients, causing half of them to drop out of the study. The drop-outs subsequently reacted better to topical corticosteroids than they had before. A second pilot study suggested that an additive treatment with topically applied corticosteroids permits lowering of the oral dose of the retinoid to a level at which side-effects are more acceptable (Polano & van der Rhee, 1978).

To test this hypothesis we designed a multi-centre double-blind trial followed by an open clinical trial, the results of which are presented in this paper.

## MATERIALS AND METHODS

### *Patients*

The study was designed as a double-blind randomized multicentre clinical trial with a duration of 6 weeks followed by an open study with a duration of 12 weeks. Twelve dermatologists at three centres* cooperated in this trial.

The subjects were male and female ambulatory out-patients with psoriasis vulgaris involving at least 15% of the skin surface, who had been unsuccessfully treated with one or more of the generally accepted therapies for psoriasis. Virtually all had been treated with potent corticosteroids with occlusive dressings and thirteen patients had also received PUVA treatment. Patients under the age of 18, pregnant women, patients with acute guttate psoriasis, and patients previously treated with aromatic retinoid were excluded. Informed consent was obtained from each patient. Ninety patients were used in the trial.

### *Experimental design*

*Double-blind study* (*duration* 6 *weeks*). Patients were randomly assigned to one of the three following treatment regimens:

(1) Aromatic retinoid and placebo cream (lanette wax cream). This regimen will be referred to as AR/Plcr.

(2) Aromatic retinoid and 0·1% triamcinolone acetonide and 5% salicylic acid in lanette wax cream. This will be abbreviated to AR/Tr.

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(3) Placebo capsules and 0·1% triamcinolone acetonide and 5% salicylic acid in lanette wax cream. This will be abbreviated to Plca/Tr.

Because it was considered unethical to assign patients to a Plca/Plcr treatment, this treatment was omitted. In each of the three centres the ratio AR/Plcr:AR/Tr:Plcr/Tr was 1:1:1.

The patients were supplied with 100 g active or placebo cream per week. The aromatic retinoid or placebo was given in 10 mg capsules according to the patient's body weight: patients weighing 40–60 kg, 60–80 kg, or 80–100 kg received 3, 4, or 5 capsules, respectively, per day. This scheme results for aromatic retinoid in a daily dosage varying between 0·50 and 0·66 mg/kg body weight, which is about half the dosage currently recommended.

*Open study.* After the double-blind study, patients were treated for 12 weeks with regimen 2, i.e. active cream and active capsules in the dosage indicated above (AR/Tr).

*Evaluation.* Patients were examined 3, 6, 9, 12, and 18 weeks after the start of the treatment. At the start (week 0) the lesions were plotted on a preprinted body diagram, and 'intensity scores' were given for the lesions of six body areas separately: scalp, arms, hands, trunk, legs, and feet, according to a grading system used in earlier clinical trials (Polano *et al.*, 1970). The scale for desquamation runs from 0 (no desquamation) to 8 (very marked desquamation). The scale for erythema and infiltration runs from 0 (no erythema) via 4 (moderate infiltration) to 8 (very marked infiltration).

To standardize scoring the physicians were given an illustrated booklet. The intensity score for the six body areas was assessed again after 3, 6, 9, 12 and 18 weeks. At this time, the overall improvement (in units of 10%) relative to week 0 was evaluated by both the physician and the patient. Generally, patients were evaluated by the same physician each time.

*Side-effects and laboratory tests.* At 3, 6, 9, 12, and 18 weeks side-effects were evaluated on the basis of the following scale: 0 = absent, 1 = mild, 2 = severe. At week 0 and week 18 the following laboratory tests were performed: complete blood count, blood sedimentation rate, blood glucose, urea, creatinine, electrolytes, bilirubin, cholesterol, albumin, serum protein, SGOT, SGPT, lactic dehydrogenase, and alkaline phosphatase; and at weeks 3, 6, 9, 12: SGOT, SGPT, lactic dehydrogenase, and alkaline phosphatase.

*Statistical analysis.* To assess the equivalence of the three groups at the beginning of the trial, the findings at week 0 were evaluated by an analysis of variance or a chi-square test. The three groups were compared simultaneously.

For assessment of the treatments, two tests were usually performed for each criterion, one to compare AR/Tr with AR/Plcr and the other AR/Tr with Plca/Tr.

The intensity scores at week 6 were analysed with the Mantel-Haenszel test, taking the corresponding intensity score at week 0 as stratum identification. In this way comparisons between the two groups are made for each initial score separately and the evidence obtained is combined (Mantel, 1963). Because of space restrictions, the data are presented as average improvement scores.

Assessment of the improvement rates at week 6 was also made with the Mantel-Haenszel method, but in this case the centres were taken as the strata.

The trial started with ninety patients, three of whom were not included in the trial. In the second part of the study another nine patients were lost either because of side-effects or due to failure to keep to the appointment schedule. Because of incomplete data in some cases, the analysis of the improvement rates in this part of the study was restricted to seventy-four patients.

TABLE 1. Means of the intensity scores at week 0 and week 6 and *P* values used to assess improvement after 6 weeks. For week 0 some general data are given too

| Treatment | AR/Plcr | | | AR/Tr | | | Plca/Tr | | | *Comparison at week 6* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Week 0 | Week 6 | ⓧ | Week 0 | Week 6 | ⓧ | Week 0 | Week 6 | ⓧ | *AR/Tr vs AR/Plcr P value** | *AR/Tr vs Plca/Tr P value** |
| Erythema and infiltration | | | | | | | | | | | |
| Scalp | 3·4 | *2·1* | *1·3* | 2·6 | *1·2* | *1·4* | 2·8 | *1·9* | *0·9* | *0·05* | *0·04* |
| Arms | 4·1 | *3·1* | *1·0* | 3·9 | *2·0* | *1·9* | 3·7 | *2·6* | *1·1* | *0·01* | *0·10* |
| Hands | 2·6 | *1·7* | *0·9* | 2·8 | *1·2* | *1·6* | 2·2 | *1·8* | *0·4* | *0·009* | *0·005* |
| Trunk | 4·1 | *3·1* | *1·0* | 3·8 | *2·0* | *1·8* | 3·6 | *2·4* | *1·2* | *0·004* | *0·54* |
| Legs | 4·3 | *3·5* | *0·8* | 4·3 | *2·3* | *2·0* | 4·0 | *2·9* | *1·1* | *0·002* | *0·04* |
| Feet | 2·4 | *1·8* | *0·6* | 2·3 | *1·6* | *0·7* | 2·3 | *1·8* | *0·5* | *0·41* | *0·76* |
| Desquamation | | | | | | | | | | | |
| Scalp | 4·3 | *2·2* | *2·1* | 2·7 | *1·3* | *1·4* | 3·5 | *1·8* | *1·7* | *0·52* | *1·00* |
| Arms | 3·2 | *2·4* | *0·8* | 3·5 | *1·6* | *1·9* | 3·3 | *1·9* | *1·4* | *0·09* | *0·45* |
| Hands | 2·1 | *1·1* | *1·0* | 2·1 | *0·8* | *1·3* | 1·8 | *1·3* | *0·5* | *0·21* | *0·02* |
| Trunk | 3·2 | *2·3* | *0·9* | 3·1 | *1·2* | *1·9* | 3·0 | *1·7* | *1·3* | *0·005* | *0·10* |
| Legs | 3·2 | *2·4* | *0·8* | 3·6 | *1·7* | *1·9* | 3·5 | *2·0* | *1·5* | *0·07* | *0·89* |
| Feet | 2·1 | *1·4* | *0·7* | 1·7 | *1·2* | *0·5* | 2·0 | *1·5* | *0·5* | *0·94* | *0·43* |
| Age (years) | 43·7 | | | 44·7 | | | 43·7 | | | | |
| Weight (kg) | 71·2 | | | 71·1 | | | 72·1 | | | | |
| Duration (years) | 20·4 | | | 20·8 | | | 22·9 | | | | |
| Sex (male–female) | 18–11 | | | 15–14 | | | 15–14 | | | | |
| Prior PUVA (yes–no) | 4–25 | | | 5–24 | | | 4–25 | | | | |

ⓧimprovement at week 6 as compared with week 0.

* computed according to the Mantel-Haenszel method.

The initial values for the three groups were compared by an analysis of variance. Comparison of the three groups at week 0 yielded, for all items, *P* values higher than 0·25 with the exception of desquamation on the scalp, which had a *P* value of 0·03.

RESULTS

*Double-blind study*

Relevant data registered upon admission to the trial are given in Table 1. By the sixth week, three patients (one in each treatment group) had dropped out of the study, one because the treatment was discontinued in another hospital after his admission because of an exacerbation of pre-existing pulmonary complaints, and the other two because of failure to keep to the appointment schedule. These three patients are not included in Table 1.

TABLE 2. Evaluation of the overall improvement by the physician and the patient at week 6 for the three treatment groups

| | Physician | | Patient | |
|---|---|---|---|---|
| | Percentage | *P* value | Percentage | *P* value |
| AR/Plcr | 18·0 | | 8·8 | |
| | | 0·0011 | | 0·0015 |
| AR/Tr | 55·4 | | 47·9 | |
| | | 0·22 | | 0·38 |
| Plca/Tr | 40·8 | | 38·0 | |

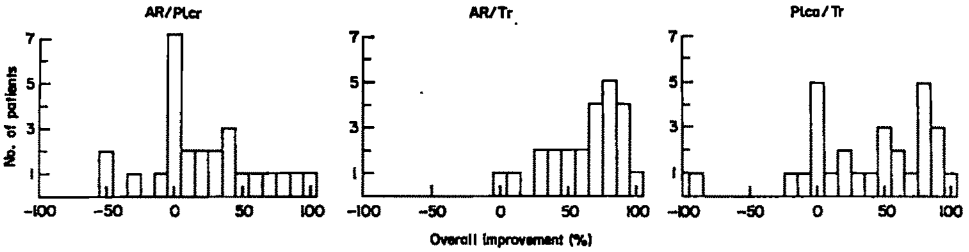

FIGURE 1. Histograms representing the overall improvement (as assessed by the physician) in percentages 6 weeks after the start of the treatment, for the three different treatment groups.

*Overall improvement.* The averages and relevant *P* values for the improvement rates as judged by the physician as well as by the patient himself are given in Table 2. Histograms of the overall improvement as judged by the physician are shown in Fig. 1. Histograms showing the patients' evaluations are not given, because they are very similar to those made by the physician. Apparently, retinoid with active cream gives significantly better results than retinoid alone. Retinoid with active cream gave better results than active cream alone, but this difference is not significant.

*Intensity score.* The intensity score at 6 weeks and the relevant *P* values are also given (in italics) in Table 1. The combination therapy AR/Tr was more effective in reducing the initial intensity score than was the aromatic retinoid alone (AR/Plcr), particularly for erythema and infiltration, for which the differences in effectiveness were statistically significant for all body areas except the feet. The AR/Tr regimen was also more effective than the active cream alone (Plcr/Tr) for all body areas and all

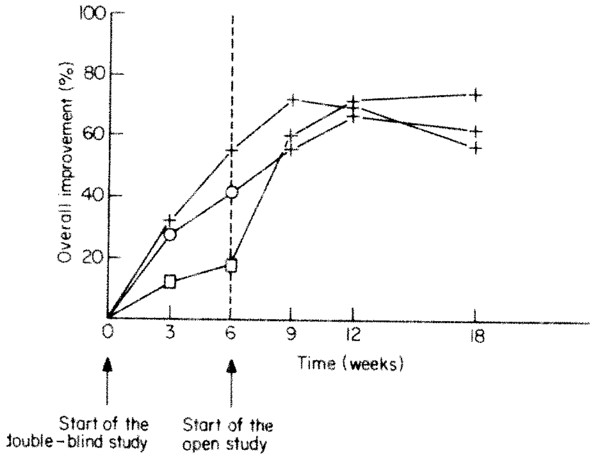

FIGURE 3. The course of the mean overall improvement (as assessed by the physician) for the three treatment groups (Note: after 6 weeks all patients were put on the same treatment). (+—+) AR/Tr; (○—○) Plca/Tr; (□—□) AR/Plcr.

symptoms (except desquamation of the scalp and feet), although with statistical significance only for erythema and infiltration of the scalp, hands, and legs and desquamation of the hands. The effect was greater for erythema and infiltration than for desquamation.

*Open study (weeks 6–18)*
After 6 weeks, all patients received the same treatment: retinoid capsules and the corticoid-containing cream (AR/Tr). At the end of the study twelve patients had dropped out because of side-effects (4) or because of failure to keep appointments (8).

*Overall improvements.* Figure 3 shows the course of the mean overall improvement of the three patient groups, which had received different treatments in the first 6 weeks of the study. The final overall improvement of all individual patients is shown in Fig. 4. Again, there were no important differences between the physician's evaluation and that of the patients. For the thirteen patients who had previously been unsuccessfully treated with PUVA, the final results were as follows: four were free of symptoms, two showed good improvement, three moderate improvement, two no change, one deterioration, and one patient was not evaluated because of failure to keep the appointment.

*Intensity score.* The differences in intensity score between the three treatment groups at week 6 disappeared after all patients were put on the same treatment. On the whole, the decline of the intensity scores corresponded with the course of the overall improvement. At the end of the study, the average score for all body areas was about 1 for erythema and 1 for desquamation, except for the legs, which on average scored 2 for these symptoms.

*Side-effects*
Table 3 shows the occurrence and degree of the main side-effects at week 6 for the three different treatment groups.

Table 4 gives the incidence of the side-effects for the open study as well as the number of visits at which these side-effects were noted for individual patients. The most common side-effect was dryness of the lips, which was generally well tolerated; the majority of the patients who suffered from it

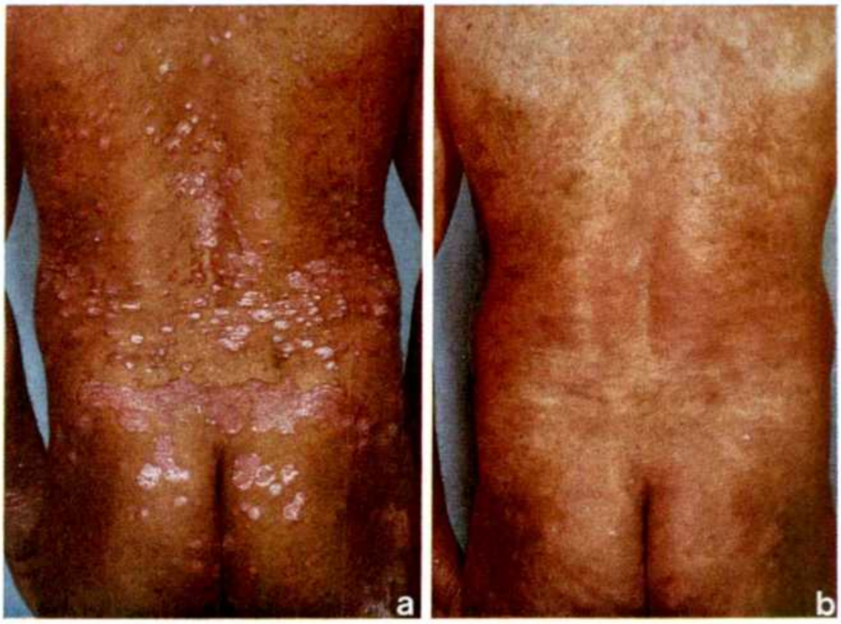

FIGURE 2. This patient had psoriasis for many years and had been treated inadequately with several therapies, including PUVA. (a) Clinical situation (intensity scores: 4 for desquamation and 5 for erythema and infiltration) at week 0; and (b) at the end of week 6 (intensity scores: 0 for desquamation and 2 for erythema) of the double-blind trial, in which he was treated with aromatic retinoid and active cream (AR/Tr).

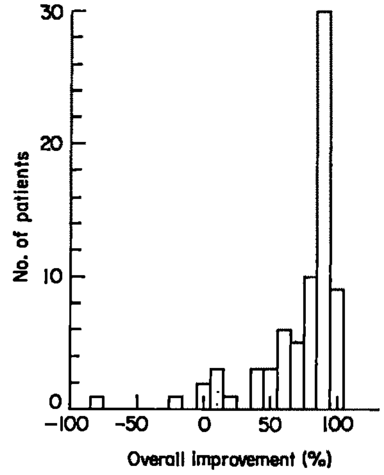

FIGURE 4. Histogram showing the final overall improvement (as assessed by the physician after 18 weeks of treatment) for all patients who completed the study.

TABLE 3. Side-effects at the end of the double-blind trial (week 6)*

| Side-effects | AR/Plcr (n = 29) | | AR/Tr (n = 29) | | Plca/Tr (n = 29) | |
|---|---|---|---|---|---|---|
| | Mild | Severe | Mild | Severe | Mild | Severe |
| Dryness of the lips | 17 | 1 | 21 | 1 | 4 | 0 |
| Rhagades | 2 | 0 | 3 | 1 | 0 | 0 |
| Dryness of the nose | 3 | 0 | 8 | 1 | 1 | 0 |
| Dryness of the eyes | 2 | 0 | 3 | 0 | 0 | 0 |
| Palmoplantar desquamation | 0 | 0 | 4 | 0 | 0 | 0 |
| Pruritus | 10 | 3 | 6 | 2 | 0 | 0 |
| Atrophy of the skin | 0 | 0 | 3 | 0 | 1 | 0 |
| Alopecia | 1 | 0 | 0 | 0 | 1 | 0 |

* All values represent numbers of patients who reported the side-effect in question as mild or severe.

did not show objective signs of cheilitis, such as desquamation and rhagades of the lips. Four patients developed bruises on the arms and legs after very mild trauma.

### *Laboratory tests*

Except in one case, the laboratory investigations revealed no significant abnormalities, and we found no significant elevation of the SGOT, SGPT, and alkaline phosphatase levels of the patients treated with the retinoid. For lactic dehydrogenase, however, we found a significant ($P<0{\cdot}05$) increase, but still within the normal range, 3 and 6 weeks after the start of the retinoid treatment; this elevation disappeared later. Only one patient showed a considerable elevation of the SGOT (72 u/l), SGPT (168 u/l), and lactic dehydrogenase (151 u/l) levels 6 weeks after the start of the therapy. The alkaline phosphatase concentration remained within the normal range. A percutaneous liver biopsy showed very mild damage of the parenchymal liver tissue and minor periportal fibrosis, i.e. grade II according

TABLE 4. Side-effects reported by seventy-eight patients for the ninth, twelfth, and eighteenth week (the open part of the study).

| | For the three visits, the side-effect was reported: | | | |
|---|---|---|---|---|
| Side-effect | Never | At one | At two | At all three |
| Dryness of the lips | 19 | 6 | 6 | 47 |
| Rhagades | 57 | 9 | 4 | 8 |
| Dryness of the nose | 54 | 9 | 5 | 10 |
| Dryness of the eyes | 70 | 3 | 3 | 2 |
| Dryness of the throat | 77 | 1 | 0 | 0 |
| Palmoplantar desquamation | 71 | 4 | 2 | 1 |
| Pruritus | 57 | 4 | 10 | 7 |
| Atrophy of the skin | 74 | 2 | 2 | 0 |
| Alopecia | 73 | 2 | 1 | 2 |
| Burning sensation on face | 76 | 1 | 0 | 1 |
| Epistaxis | 76 | 1 | 1 | 0 |
| Bruises on arms and legs | 74 | 1 | 3 | 0 |
| Generalized erythema | 75 | 2 | 1 | 0 |
| Paronychia | 77 | 1 | 0 | 0 |

to the MTX schedule of the Task Force of the A.A.D. On this basis, the treatment was continued and within 3 months the levels gradually returned to normal.

Extensive coagulation tests performed on the patients who showed bruises on arms and legs did not reveal abnormalities.

## DISCUSSION

In the present study it was found that the new aromatic retinoid Tigason given in low dosage orally (0·50–0·66 mg/kg body weight), in combination with triamcinolone acetonide (0·1%) cream with 5% salicylic acid, offers an effective treatment for severe psoriasis vulgaris, side-effects being mild.

This first double-blind trial has shown that, in the dosage used, retinoid alone has no effect at all. The combined treatment gave better results than the triamcinolone/salicylic acid treatment alone. This effect, which was apparent for almost all variables, could only be proven statistically for some of them. Since the active cream alone could be expected to have a considerable impact on desquamation, the absence of a strong effect after the addition of retinoid is not surprising. The effect on erythema and infiltration (see Table 1) is more striking.

In this trial the effectiveness of the cream containing 0·1% triamcinolone acetonide and 5% salicylic acid is remarkable (mean overall improvement in 6 weeks: 40%), especially in view of the fact that these patients had previously obtained little or no benefit from various strong corticoisteroids topically, often in combination with salicylic acid.

As can be concluded from Fig. 3, the maximal result obtained in the three treatment groups could be maintained to varying degrees up to week 18. After follow-up for 1 year, the majority of the patients who had responded well in the present study still showed good results (van der Rhee *et al.*, unpublished data). These long-term results are, in our opinion, superior to those generally seen after long-term treatment with corticosteroids.

A similar long-lasting effect has been found for a combination of the aromatic retinoid with dithranol

(Orfanos & Goerz, 1978), although in these studies a high initial dosage of the retinoid was used. The new aromatic retinoid in high dosage has also been combined with other psoriasis therapies, such as PUVA (Fritsch *et al.*, 1978; Orfanos *et al.*, 1978a), dithranol (Orfanos & Runne, 1976; Orfanos & Goerz, 1978), and selective ultraviolet therapy (Steigleder, Orfanos & Pullmann, 1978), and in these cases too the combination was more effective than one of the constituents alone.

At the end of the open study 60% of the patients had obtained a 80–100% clearance. This is more or or less similar to the results obtained with monotherapy with this retinoid in the usual high dosage (Ott, 1977; Orfanos & Goerz, 1978; Orfanos *et al.*, 1978b).

### *Side-effects*

It is clear from Table 4 that the present combination causes relatively few side-effects compared with the usual high dosage, as also shown by our earlier results (see Introduction) and those described in several reports in the literature (e.g. Ott & Bollag, 1976; Orfanos *et al.*, 1978b; Orfanos & Goerz, 1978; Fritsch *et al.*, 1978). Fritsch *et al.* (1978) also found that lowering of the dose causes fewer side-effects. Although the percentage of patients who develop dry lips is similar to those given in the above-mentioned reports, our patients generally did not have clinical cheilitis with desquamation and rhagades. Furthermore, complaints of dryness of other mucosae were negligible. None of the patients suffered severe hair loss, the main reason given for interrupting high-dosage retinoid therapy in other studies.

Side-effects known to be caused by topical corticosteroids were not observed. A few of the patients showed atrophy of the skin on the distal parts of arms and legs; this too has been reported by several authors after monotherapy with this retinoid. No striae were observed.

The plasma cortisol levels which were incidentally estimated in some of the patients were within normal range. No liver dysfunction or other systemic side-effects were found during intensive screening.

In sum, we can say that the combination of low-dosage aromatic retinoid orally with 0·1% triamcinolone acetonide acid and 5% salicylic acid in a cream is a potent treatment for psoriasis, at least as effective as retinoid monotherapy in the usual dosage of 1 mg/kg body weight and giving markedly fewer side-effects.

## ACKNOWLEDGMENTS

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# Treatment of disseminated superficial actinic porokeratosis with a new aromatic retinoid (Ro 10-9359)

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## SUMMARY

An oral aromatic retinoid Ro 10-9359 controlled the skin symptoms and the pruritus of a patient with disseminated superficial actinic porokeratosis (DSAP).

Recently, aromatic retinoids have been successfully used for the treatment of various skin diseases characterized by epidermal hyperproliferation and disturbed keratinization (for reviews, see Orfanos & Schuppli, 1978). Thus, oral retinoids seem to be effective in the treatment of psoriasis, palmoplantar pustulosis, Darier's disease, lamellar ichthyosis and pityriasis rubra pilaris.

## CASE REPORT

A detailed report of our patient has been published earlier (Kariniemi, Kotovirta & Stubb, 1979). The patient, an 84-year-old Finnish woman, had suffered since 1976 from a highly pruritic dermatosis on the trunk and extremities. The initial lesions were small red papules, which grew to form brownish plaques surrounded by a thread-like scaly collar. A skin biopsy taken from the arm revealed typical cornoid lamellae with parakeratotic columns at both edges of the plaque. Marked solar elastosis of the dermal collagen fibres was noted. The eruption was extremely pruritic, greatly disturbing the life of the patient and it had been resistant to all previous therapy.

The administration of an aromatic retinoid (Ro 10-9359, all-trans-9-(4-methoxy-2,3,6-trimethylphenol)-3,7-dimethyl-2,4,6,8-nonatetraenoate, F. Hoffman-La Roche & Co. A.G.), 100 mg daily, was started in September 1978. Within 10 days the itching began to decrease and the lesions to fade. The dose was reduced to 50 mg daily. After 40 days' treatment the pruritus had ceased entirely and the lesions had cleared so that the scaly thread-like border had disappeared. The dose was now reduced to 25 mg daily. A further skin biopsy was taken from the most prominent lesion on the arm after 2 months of therapy. The cornoid lamella was still visible, but it was small and shallow. Since February

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1979 the patient has taken 25 mg of retinoid every other day. She experiences itching only occasionally and the lesions are hyperpigmented but otherwise only slightly apparent.

The following laboratory tests were performed during the treatment period: complete blood cell count, erythrocyte sedimentation rate, urinalysis, fasting blood sugar, SGOT, SGPT, alkaline phosphatase, serum bilirubin, and serum creatinine. No abnormalities were found. The side-effects of the medication were slight, a mild cheilitis and hair loss, which decreased when the drug dosage was reduced. After 6 months of treatment the patient developed a peculiar follicular hyperkeratosis with tiny keratin horns on the skin of both forearms.

## COMMENT

DSAP, an autosomal, dominant, hereditary genodermatosis, has been suggested to be due to hereditary mutant cell clones in the epidermis (Reed & Leone, 1970). These cells are sensitive to ultraviolet light, which causes the disturbed keratinization.

Vitamin A acid has been used locally for the treatment of DSAP without any effect (Gutschmidt, 1977). An oral aromatic retinoid (Ro 10-9359) induced remarkable improvement of the disease in our case. The mechanism of action of the aromatic retinoid is unknown. Like vitamin A and its other derivatives it probably participates in the differentiation of the epidermal cells (Bollag, 1974; Orfanos *et al.*, 1979). It seems to enhance keratinization, leading to reduction of the mitotic activity. According to the histological findings in our case Ro 10-9359 seems to act in DSAP primarily on the mutant parakeratotic cells at the cornoid lamellae, rectifying the keratinization and clearly also inhibiting the proliferation of these cells.

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***Case Reports***

# Botryomycosis: a complication of extensive follicular mucinosis

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## SUMMARY

A case of extensive follicular mucinosis, without evidence of lymphoma and present for 9 years, was complicated by cutaneous botryomycosis which initially resembled sporotrichosis. Scanty *Staphylococcus aureus* was cultured with difficulty.

No immunodeficiency could be demonstrated, and the importance of a local tissue factor (namely follicular mucinosis) in the production of the characteristic histological reaction of botryomycosis is emphasized.

Botryomycosis (also botriomycosis; Greek, *botrys*, a bunch of grapes) was first reported in 1870 by Bollinger in a horse's lungs. He described the case as one of chronic pneumomycosis and in 1888 proposed that the causal organism be named 'botryomyces' believing it to be a fungus. Rivolta (1884) was the first to describe the disease as botryomycosis. Opie (1913) reported the first human case from the U.S.A., and the subject was fully reviewed in 1959 by Winslow. He found only forty human cases in the literature and added six others from the records of the Armed Forces Institute of Pathology.

Botryomycosis is characterized by chronic suppurative, granulomatous lesions in which the diagnostic feature is the presence of grains (Bollinger's granules) resembling those of actinomycosis or mycetoma within purulent foci. These grains contain swollen cocci, which, when stained with Gram or Giemsa stains, resemble the bunches of grapes after which the disease is named. The condition is common in animals but rare in man, and a search of the literature—including a Medlar search—has revealed no previous reports of it as a complication of follicular mucinosis.

## CASE REPORT

The patient was a Welshman, aged 60, employed as a caterer/chef. He drank 6–12 pints of beer a day and had been a heavy smoker until 1976. There was no relevant family history.

In 1969 he developed a rash on the forehead which spread to the chin, eyebrows and limbs. He

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was seen by dermatologists in Australia where he worked for 7 years, at times catering for a mining community. A diagnosis of follicular mucinosis, supported by histology, was made. At the time, full blood count and E.S.R. were normal. Liver and spleen scans showed marginal hepatomegaly. Bilateral blepharitis was thought to be due to the widespread follicular mucinosis. Treatment with steroid creams helped a little.

In December 1976 the patient returned to the United Kingdom and in February 1977 was seen at hospital with extensive follicular mucinosis, with areas of discoloration, and loss of hair of eyebrows, face, scalp, all limbs and pubic region. There were many small sebaceous cysts of the eyelids. These were excised and biopsies of the neck and left lower cheek were carried out. The histology showed the changes of follicular mucinosis and there was no evidence of malignancy. He was treated with clobetasone butyrate 0·05% cream (Eumovate) with excellent results until August 1977, when a small number of indolent, inflammatory nodules on the right wrist and hand appeared (Fig. 1). A biopsy of a nodule was taken and the histology was reported as showing tuberculoid granulomatous abscesses typical of sporotrichosis. The clinical picture was entirely consistent with this diagnosis, including a linear pattern of inflammatory nodules indicating lymphatic spread up the forearm. A report of the presence of *Sporothrix schenckii*, the causal organism of sporotrichosis, in cold-stored frankfurters (Ahearn & Kaplan, 1969) lent credibility to this diagnosis in a chef. Later new lesions erupted on both arms and legs, and a diagnosis of disseminated, cutaneous sporotrichosis in a potentially immunodepressed

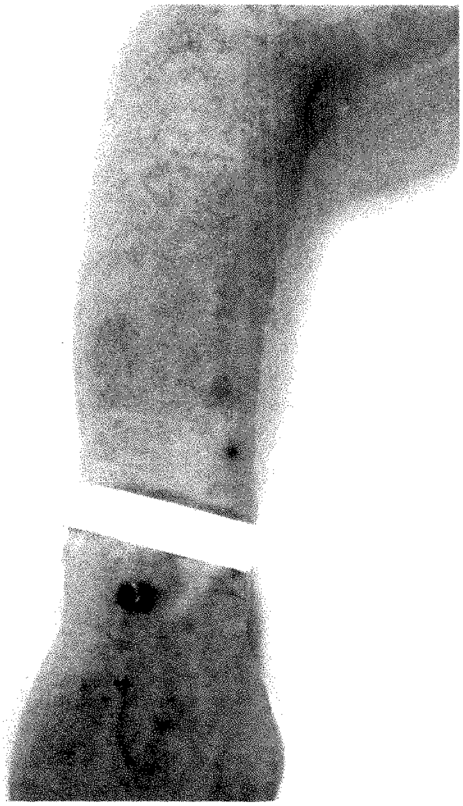


FIGURE 1. Right forearm and wrist of patient showing sheets of follicular mucinosis and sporotrichoid pattern of lesions of botryomycosis.

subject was entertained. Tied by his Christmas duties, the patient refused immediate admission to hospital and he was given a low dose of potassium iodide as an out-patient (0·6 ml of the saturated solution three times daily). However, two successive cultures of pus failed to yield *S. schenckii*, and counter immuno-electrophoresis and indirect fluorescent antibody tests on his serum, carried out at the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine, failed to demonstrate antibodies to the fungus.

The lesions became larger, more numerous and involved the pubic region, and a further biopsy was carried out. Half of the tissue was submitted to histology and half was cultured for fungi. The histology was reported as being identical to that seen previously, probably sporotrichosis, but cultures for fungus were again unsuccessful.

After Christmas the patient was admitted to Bristol Royal Infirmary and further investigations were carried out. The following tests gave normal or negative results: full blood count, blood viscosity, blood cultures, N.B.T. test, auto-immune profile, bone marrow aspiration, urinalysis and chest X-ray. Liver function tests, however, were abnormal. Plasma proteins 43 g/l, IgA 6·3 g/l, IgG 23·7 g/l, IgM 0·34 g/l. Aspartate-amino transferase 21 iu/l.

*In vitro*, lymphocyte tests showed that the T-lymphocyte population was depressed (cells rosetting with sheep red blood cells $0{\cdot}93 \times 10^9$/l, 37% of the total lymphocyte count) with a corresponding depression of the P.H.A. transformation index. The latter was considered to be partly due to high counts in the unstimulated cultures which could reflect a response to circulating active lymphocytes. It was suggested that the test should be repeated after treatment had been completed.

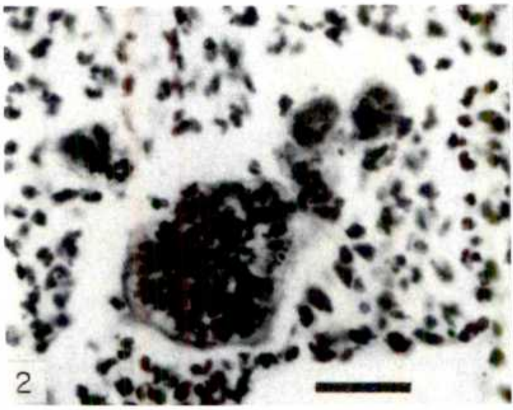
### *Progress*

On admission, the dose of saturated solution of potassium iodide was gradually increased to 3·0 ml three times daily. The patient's condition rapidly grew worse and the mucinosis lesions became very erythematous. Widespread pustular folliculitis developed from which large quantities of *S. aureus* were isolated, and multiple new nodular lesions appeared. From pus aspirated from the latter, very small quantities of *S. aureus* were isolated, but as it had the same sensitivities as that from the follicles, it was thought that the organism was a contaminant from the follicular pustules. Iodism became severe and potassium iodide treatment was stopped.

At no time was it possible to isolate *S. schenckii* from the lesions, and a skin test for sporotrichosis which was carried out at this time, with sporotrichin kindly supplied by Dr F.Mariat, Institut Pasteur, Paris, was negative. The biopsy sections were, therefore, re-examined.

### *Comparative histopathology*

A small, but otherwise characteristic, grain from the patient is illustrated in Fig. 2 and an asteroid of sporotrichosis in Fig. 3. While both structures are surrounded by a thick, amorphous deposit, the asteroid (about 20 μm in diameter) is much smaller than the grain (about 60 μm, many were larger). The asteroid contains a single, budding yeast about 7 μm in diameter, while the grain contains a number of smaller, Gram positive, spherical objects 1·0–2·0 μm in diameter that are nevertheless larger than bacteria (up to 1 μm). They show no budding. They were also positive to periodic acid-Schiff and Grocott stains. It was concluded that the histology of the biopsy was not compatible with that of sporotrichosis. Comparison of the patient's sections with those from a case of equine subcutaneous botryomycosis due to *S. aureus* (Fig. 4) showed them to be very similar, and *S. aureus*, even though in small quantities, had been isolated from the patient's lesions. It was concluded that the patient's lesions were due to staphylococcal botryomycosis, a rare condition in man. The granulomatous reaction was thought, possibly, to reflect an abnormal immunological state.



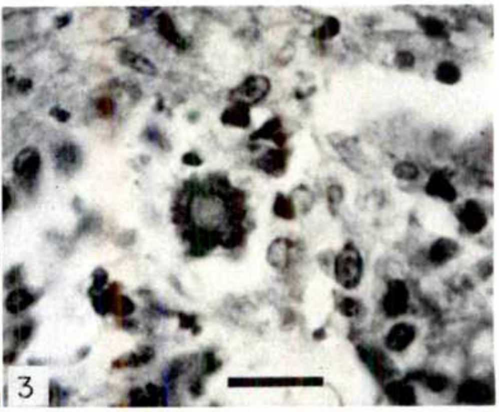


FIGURE 2. Botryomycotic grain from a lesion of the patient's arm. The grain contains numerous swollen bacteria most of which have taken the Gram stain, though a few are Gram-negative. There are no peripheral clubs. Bar = 20 μm. FIGURE 3. Asteroid of sporotrichosis in man, containing a single, budding yeast cell. For comparison with Fig. 2, bar = 20 μm.

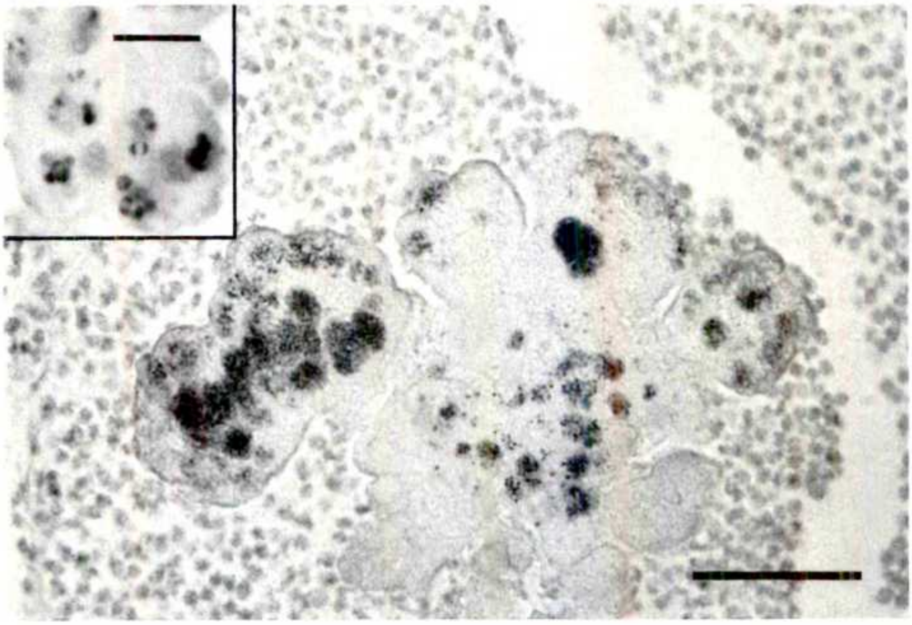


FIGURE 4. Botryomycotic grains in the horse. The lobulated grains are without peripheral clubs and show Gram-positive *Staphylococcus aureus* in scattered or clustered arrangement inside. Bar = 50 μm. Inset: higher magnification to show unstained, partly stained and fully stained staphylococci. Bar = 10 μm.

### *Further progress*

Treatment with phenoxymethylpenicillin 250 mg four times daily was begun, and 3 days later the patient's pustular lesions and his whole condition had dramatically improved. Penicillin treatment was continued for 3 weeks, and by April 1978 almost all the pustular lesions had resolved. In June there were a small number of follicular pustules, but the patient was otherwise well, apart from his extensive follicular mucinosis.

In July 1978 further immunological tests were carried out with the following results: Antistaphylolysin, 2–4 units/ml—slightly raised titre. Candida antigen skin test positive. Streptokinase/streptodornase test negative—immediate and delayed responses. Mantoux test positive at 1:1000. T and B lymphocytes normal. T-cell count (Srbc rosettes) $1{\cdot}08 \times 10^9$/l. B-cell count (surface bound immunoglobulin) $0{\cdot}3 \times 10^9$/l.

## DISCUSSION

Botryomycosis is a total misnomer for this disease for it is not of fungal origin and there are no fungi designated as 'botryomycetes'. Although lengthy and less arresting, the name 'bacterial pseudomycosis' (Greenblatt *et al.*, 1964) is more accurate. 'Actinobacillosis', 'staphylococcal actinophytosis' (Berger, Vallée & Vezina, 1936; Waisman, 1962) and 'granular bacteriosis' (Winslow, 1959) have also been used. Even greater confusion existed when pyogenic granuloma (granuloma telangiectaticum) was also dubbed 'pseudobotryomycosis' or 'botryomycosis hominis'. The reason for this confusion was given by M'Fadyean (1919) as a failure to recognize the essential microscopical features of the granules of botryomycosis. The French literature introduced further disorder by referring to 'cutaneous epithelioma of the botryomycoma type' (Bureau *et al.*, 1969, 1970). Botryomycosis was described as a disease entity with a specific histology easily confused, pathologically, with granulomatous fungal disease and, as in this case, it still tricks the pathologist and clinician. For this reason the name is worth keeping.

The usual conditions simulated by the pathological appearances are actinomycosis (Fig. 5), mycetoma due to both fungi and actinomycetes, and sporotrichosis. In all of these, as in botryomycosis, the deposit surrounding the colonies or individual cells of the pathogen is an antigen–antibody precipitate (Symmers, 1974; Lurie & Still, 1969), probably also involving other metabolites of the pathogen (Findlay & Vismer, 1974). Occasionally, round ova or dead metazoan parasites may be thought present (Symmers, 1973) and a case complicating larva migrans (*Ankylostoma caninum*) has been reported (Privat *et al.*, 1968). A curious case resembling Maffucci's syndrome was described by Waisman (1962).

### *Diagnosis*

The important points to note in making a diagnosis of botryomycosis due to *Staph. aureus* are as follows.

*Histology.* Grains associated with bacterial, actinomycete and fungal infections consist of an amorphous, eosinophilic, 'zoogloeal' deposit surrounding a single cell to a whole colony of the organism. In botryomycosis the grains contain numerous round, swollen bacterial cells which measure 1·0–2·0 μm in diameter. Living cells stain with Gram, periodic acid-Schiff and Grocott stains, but many will be dead and will fail to stain with Gram. Budding does not occur and there are no filaments or clubbing. Since clubbing in animal infections is dependent upon the animal species affected, its absence here may be a characteristic of human infection.

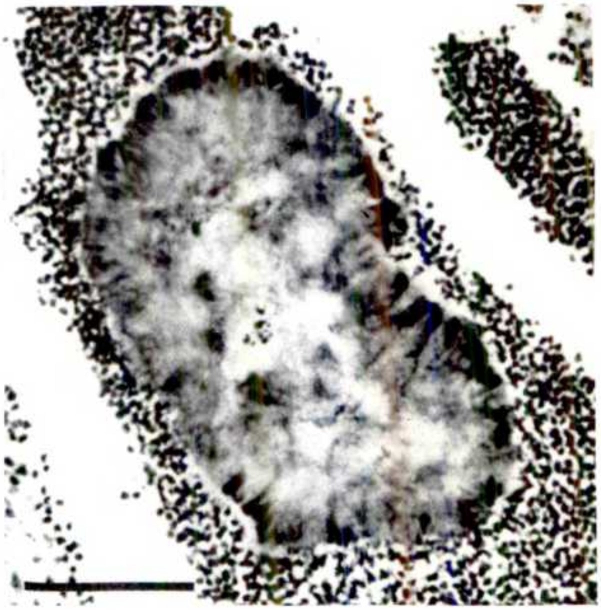


FIGURE 5. Actinomycotic grain in man showing peripheral clubbing and absence of swollen bacteria. For comparison with Fig. 2, bar = 160 μm.

*Culture*. Growth of cocci may be scanty, as in our case, unless the grains are smashed before inoculation. Cultures for fungi and actinomycetes will be negative.

*Serology*. There may be raised titres of agglutinating anti-staphylococcal antibodies in the serum. Fungal serology will be negative.

*Clinical*. The patient will respond promptly to an appropriate systemic antibacterial antibiotic. In this case, in which the initial clinical picture resembled the lymphatic spread of cutaneous sporotrichosis, it later became clear that the very large number of disseminated skin lesions, especially concentrated in areas of follicular mucinosis in a fit and rotund patient, were not consistent with a diagnosis of sporotrichosis. Such widespread cutaneous lesions could only have occurred from haematogenous spread of *S. schenckii* from multiple systemic lesions in an ill and cachectic patient.

### *Botryomycosis in animals*

The condition is seen in horses (scirrhus cord), cattle and pigs, mainly as a complication of castration. It has also been reported in sheep (Aynaud, 1928) and camels (Archibald, 1910). The lesions are intradermal, chronic, localized or spreading, and there may be extension to lymph nodes and internal organs. Similar lesions in animals have been produced experimentally. The so-called scirrhus cord in horses, which forms in connection with the spermatic cord, may weigh 7–8 lb, and even larger 'tumourous' lesions may form near the point of the horse's shoulder (M'Fadyean, 1919).

### *Human botryomycosis*

Cases of human botryomycosis fall into two groups: integumentary with or without muscle and skeletal involvement, and visceral (Winslow, 1959; Bagby & Gunning, 1978). The first group is by far the most frequent. Although *Staph. aureus* is the commonest cause, *Escherichia coli*, *Actinobacillus* (Langeron, 1941), *Pseudomonas* (Bishop, Greer & Horwitz, 1976) and *Bacillus proteus* (Winslow, 1959) have also been incriminated.

All previous authors speculate on the cause of this unusual reaction to bacteria. Suggestions include a delicate balance between the virulence of the infecting organism and the tissue resistance of the host (Magrou, 1919; Martin-Pascual & Garcia-Perez, 1975), a relative decrease in the virulence of the organisms and an increase in the resistance of the host (Drake, Sudler & Canuteson, 1943), and low virulence of the culpable staphylococcus; high titres of antistaphylococcal antibodies in the serum and inflammatrory exudate causing agglutination of cocci in clumps, but allowing survival and multiplication (Symmers, 1974); defective cellular immunity (Buckley *et al.*, 1968), and a critical low dosage of infecting organisms; a local tissue factor which confines the disease to specific anatomical types of skin. Analogous situations would be acne conglobata, hidradenitis suppurativa and dissecting cellulitis of the scalp (Waisman, 1962). The absence of any convincing specific cellular or humoral immuno-incompetence in this case of follicular mucinosis (as indeed in other cases in the literature except one patient reported by Buckley *et al.* (1968) who also suffered from chronic mucocutaneous candidiasis) makes the last suggestion of a local skin tissue factor possibly involving isolated areas of skin very attractive. The most heavily involved areas in this case were certainly those of clinically severe mucinosis, where there was hair loss, infiltration and replacement of sebaceous glands and outer root sheaths of sebaceous glands with acid mucopolysaccharides, as originally described by Pinkus (1957). There was no evidence of lymphoma.

## ACKNOWLEDGMENTS

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# Pyoderma gangrenosum complicating non-Hodgkin's lymphoma

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## SUMMARY

A case of pyoderma gangrenosum which occurred in a patient with non-Hodgkin's lymphoma is described. The lesion started in the foot where dye was introduced for a lymphangiogram.

The recognized associations of pyoderma gangrenosum are with ulcerative colitis, Crohn's disease, myeloma, leukaemia and active chronic hepatitis (Byrne, Hewitt & Summerly, 1976). There have been reports also of this disorder occurring with chronic intravascular coagulopathy (Staughton & Copeman, 1976), agnogenic myeloid metaplasia (Callen, Dubin & Gehrke, 1977), acute myeloproliferative disease (Toolis, Parker & Barnetson, 1978) and in immunosuppressed individuals (Haim *et al*., 1973). There has been one report of its occurrence in a patient with Hodgkin's lymphoma (Michigan Dermatological Society Transactions, 1974).

## CASE REPORT

A 47-year-old male printer was noticed to have considerable splenomegaly and was admitted under the general physician for further investigation. He had felt generally unwell for several weeks; otherwise there was no significant past medical, family or drug history. On examination he appeared anaemic and had a firm spleen which was palpable 15 cm below the costal margin. There was no lymphadenopathy.

Investigations showed the following: Hb 8·8 g/l, WBC 0·7 × $10^9$/l (70% lymphocytes), platelets 51 × $10^9$/l. A normal sized liver and large spleen were seen on radio-isotope scanning. A bone marrow biopsy showed mild infiltration with cells of the lymphoid series. A lymphangiogram showed abnormal para-aortic nodes.

The following investigations were normal: blood culture, brucella antibody screen, MSU, prothrombin time, chest X-ray and Paul-Bunnell.

Three days after the lymphangiogram an area of inflammation appeared on the dorsum of the left foot. It seemed to start at the region of entry of the radio-opaque dye. Swabs from the area were sterile; however, he was given antibiotics empirically. As the lesion continued to increase rapidly

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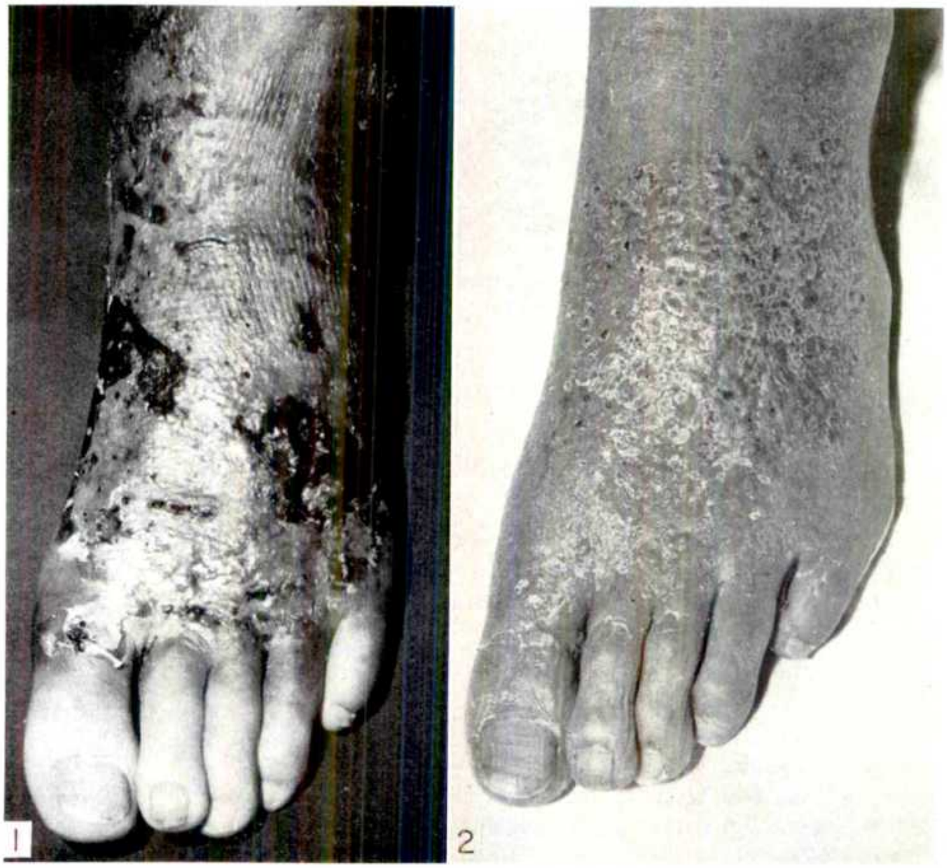
in size despite treatment with ampicillin and cloxacillin, cephazolin, metronidazole and tobramycin, a dermatological opinion was sought.

At its height the lesion measured 10 cm in diameter and the foot was extremely tender. The central area was studded with punctate haemorrhagic spots and there was an incomplete blistering rim which merged into erythema spreading for a further 5 cm (Fig. 1). During this period he had a swinging pyrexia up to 39·5°C.



FIGURES 1 AND 2. Appearance of the lesion before (Fig. 1) and 48 h after (Fig. 2) starting oral steroid therapy.

A clinical diagnosis of pyoderma gangrenosum was made, and he was started on prednisolone 30 mg daily. A skin biopsy showed a profuse mixed inflammatory infiltrate in the dermis, consistent with the diagnosis. Within 12 h the patient was pain-free and afebrile. The lesion rapidly became less inflamed and started to heal (Fig. 2). A splenectomy was performed in order to achieve a histological diagnosis and because of continuing hypersplenism. The histology of the spleen showed follicular centre cell non-Hodgkin's lymphoma. His overall condition improved following splenectomy and the pyoderma remained quiescent as the prednisolone dose was lowered gradually to 10 mg daily. Anti-lymphoma chemotherapy was started using chlorambucil.

## DISCUSSION

It is unlikely that extravasated dye could produce such an aggressive inflammatory disorder. Both the dyes used, methylene blue (to delineate the lymphatics at the start of the procedure) and Lipiodol (the radio-opaque material), are said to produce little in the way of tissue reaction. It seems more probable that trauma to the skin produced by the lymphangiogram led to pyoderma gangrenosum in a susceptible individual.

Although the bullous component was not pronounced in this case, it resembles closely the variant of bullous pyoderma described by Perry & Winkelmann (1972) and Crow & Bowers (1974). Acute leukaemias were found in patients in both these reports and trauma was clearly a precipitating factor in three of the eleven reported cases.

It seems, therefore, that patients with myelo- and lympho-proliferative disorders are at risk with regard to pyoderma gangrenosum. Any area of 'cellulitis' failing to respond to antibiotics and particularly following trauma such as lymphangiography should be examined carefully with this in mind.

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# Splenic infarction and tissue accumulation of crystals associated with the use of clofazimine (Lamprene; B663) in the treatment of pyoderma gangrenosum

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## SUMMARY

A male patient aged 68 years, suffering from pyoderma gangrenosum which was resistant to conventional treatment, received clofazimine 400 mg daily for 5 months, then reducing to 300 mg daily for the next 6 months. Eleven months after starting the drug, he was admitted to hospital with severe abdominal pain, laparotomy revealing infarction of the spleen, with violaceous congestion of the small bowel. The spleen was removed and post-operative recovery was satisfactory. Histopathological examination of the spleen (removed at operation) and of tissue from a duodenal biopsy (taken post-operatively) showed large numbers of striations and outlines suggestive of crystal deposition. Mesenteric lymph node revealed a massive accumulation of crystals in cortical and medullary sinuses. The findings emphasize that clofazimine should not be used in high dosage over prolonged periods of time, except under close clinical and laboratory supervision, and for conditions not amenable to other drugs.

Clofazimine (Lamprene; B663) was first synthesized during a programme of research for new anti-tuberculosis agents, in the laboratories of the Medical Research Council in Dublin (Barry *et al.*, 1957). It is a riminophenazine dye (2-*p*-chloranilino-5-*p*-chlorophenyl-3: 5-dihydro-3-isopropyl-iminophenazine) which forms dark red crystalline prisms; it is insoluble in water, slightly soluble in ethanol, and soluble in dioxan, dimethyl sulphoxide and methyl cellosolve. It has considerable anti-tuberculosis action in animals, but not in man, and has found its main place in the treatment of leprosy. Although possessing the serious drawback of reddish-brown pigmentation of the patient's skin (and of the lesions, in leprosy), it has nevertheless been tried and found valuable in various conditions other than leprosy including discoid lupus (Mackey & Barnes, 1974) and pyoderma

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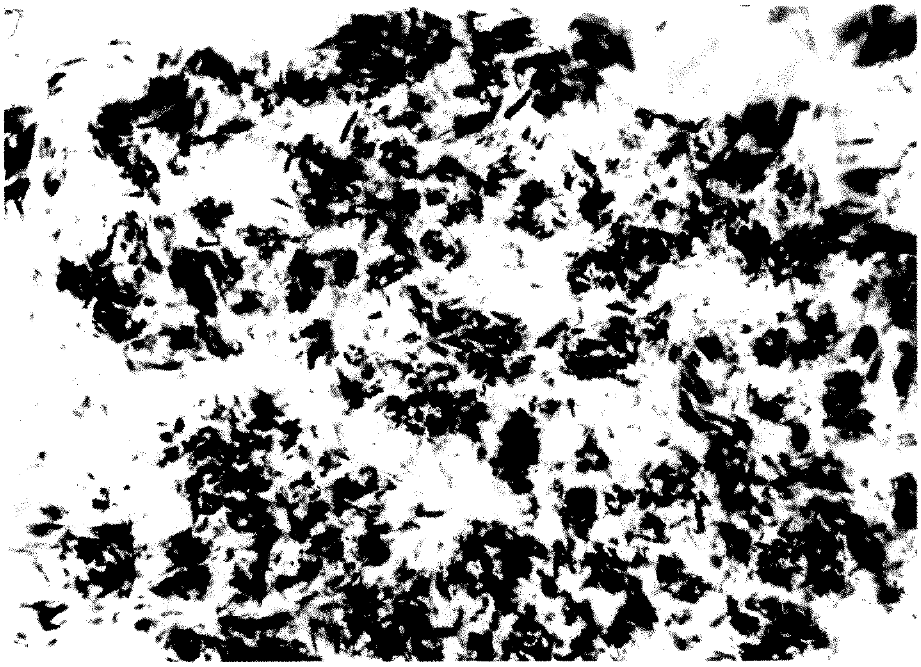

FIGURE 1. Mesenteric lymph node, removed at laparotomy for splenic infarction. Large accumulations of crystals are seen in the cytoplasm of macrophages in cortical and medullary sinuses. Fixation: 10% formalin. The sections were cleared in turpentine oil, stained with 1% aqueous methylene blue and photographed immediately (original magnification ×400).

gangrenosum (Michaëlsson *et al.*, 1976; Brandt *et al.*, 1977). We report a patient with this condition whose treatment with clofazimine over a period of 11 months was complicated by the occurrence of splenic infarction, large numbers of red crystals being demonstrated in a mesenteric lymph node taken at laparatomy.

## CASE REPORT

A male patient aged 68, with severe pyoderma gangrenosum which had failed to respond to other measures, started clofazimine 400 mg daily in July 1976, and within a few weeks showed considerable improvement. In December of that year the dose was reduced to 300 mg daily, and he remained on that dose, his skin disease being reasonably controlled, until June 1977, when he was admitted to hospital with severe left upper abdominal pain. There was no previous history of abdominal symptoms, or surgery. Pre-operative endoscopy showed unusual violaceous changes in the mucosa of the duodenum. A provisional diagnosis of acute mesenteric vascular pathology was made and laparotomy performed. The spleen was found to be enlarged and congested, with numerous fresh infarcts. The small gut was congested and had a peculiar violaceous colour, but no mesenteric vascular abnormality was present; the mesenteric lymph nodes were similarly congested and violaceous in colour. The spleen was removed, together with an upper mesenteric lymph node. Post-operative recovery was uneventful. Histopathological examination of the spleen and lymph node (in Australia) showed a heavy infiltration by histiocytes, in the cytoplasm of which there were outlines and striations suggestive of crystal deposition. In unstained sections of lymph node there were massive accumulations of red crystals (Fig. 1). A piece of splenic tissue was dissolved in 70% alcohol and compared, by spectrophotometry, with the contents of a clofazimine capsule, similarly dissolved. The results were identical with peaks at 492 nm and 290 nm.

At a later date in Oxford, cautious de-paraffinization of lymph node sections with turpentine, followed by rinsing in water, counterstaining with 1% methylene blue and mounting in turpentine, revealed that crystals lay in macrophages of cortical and medullary areas (Fig. 1). Those in the cortex tended to surround follicles (which were themselves normal), and to track down into medullary sinuses. Apart from macrophages, no other abnormal infiltrating cells were seen, and eosinophils were not in evidence in any of the three tissues examined. In the post-operative period, the findings in spleen and lymph node in Australia suggested that heavy intestinal deposition and absorption of clofazimine might have taken place, and a duodenal biopsy was therefore taken by repeat endoscopy. The histopathology showed moderate numbers of histiocytes in the lamina propria, with striations and outlines suggesting the presence of crystals. However, neither in the unstained nor in the fully processed sections of duodenum was the presence of crystals definitely confirmed, using direct or polarized light.

About 6 weeks after operation, the patient was transferred to another hospital for convalescence. Shortly after admission, he suddenly collapsed and died of cardiac arrest. ECG and other cardiological data were not available and an autopsy was not performed.

## DISCUSSION

Clofazimine was first used in the treatment of leprosy by Browne & Hogerzeil (1962) and has proved outstandingly successful in the treatment of this disease, both for the bacillary infection, including dapsone-resistant cases, and for the suppression of immune complex reactions, of which erythema nodosum leprosum (ENL) on the skin is a frequent manifestation. Despite the remarkable accumulation of crystals which is known to occur in the tissues of patients receiving clofazimine, its overall safety in clinical use is now well established (Hastings, Jacobson & Trautman, 1976). A recent review (Jopling, 1976) of some of the complications which may occur with high dosage has, however, confirmed that similar deposition of crystals may occur in the gut, spleen and lymph nodes and in 1977, Harvey *et al.* reported a case of abdominal pain and malabsorption with widespread intra-abdominal deposition of this drug, which ultimately proved fatal. We have, however, been unable to find a previous case in which splenic infarction was associated with the ingestion of clofazimine, leading to laparotomy. In the present case, the findings strongly suggest that excessive accumulation of crystals occurred in the tissues, and that this may have been related to the use of this drug over a period of 11 months, in too high a dose. It should usually be used, as the manufacturers repeatedly advise, in doses in the order of 100 mg three times weekly. Higher doses than this should be employed only under close clinical and laboratory supervision, for short periods of time, and for exceptional conditions not amenable to other drugs.

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# The treatment of pyoderma gangrenosum with sodium cromoglycate

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## SUMMARY

The successful treatment of patients with pyoderma gangrenosum by means of the local application of sodium cromoglycate solution is reported.

One of the patients also had chronic active hepatitis and a background history of ulcerative colitis. Possible mechanisms underlying the apparent success of this form of treatment are discussed.

Pyoderma gangrenosum (P.G.) may occur as a complication of ulcerative colitis although it may also be associated with Crohn's disease, chronic active hepatitis and other systemic conditions. Occasionally it occurs in the absence of identifiable underlying disease, but in those patients a coincidental history of gastro-intestinal disturbance is common (Perry & Brunsting, 1957). In general the progress of the skin lesion reflects the activity of the underlying disorder. We report two cases of P.G., which responded to the local application of sodium cromoglycate (S.C.G.) alone.

## CASE REPORTS

### *Patient* 1

A 58-year-old woman was admitted with a 5-month history of malaise, arthralgia and weight loss. A painful ulcer increasing rapidly in size had developed on her left thigh 6 weeks before. Three years earlier, an episode of bloody diarrhoea had been treated successfully with sulphasalazine and steroid enemas. Sigmoidoscopy then showed mild proctitis, and biopsy of the rectal wall subepithelial oedema and thin infiltration with polymorphs. There had been no intestinal disturbance in the intervening period.

On examination she had palmar erythema and a palpable liver edge. There was an ulcerated lesion characteristic of P.G. on the left thigh measuring 15 × 10 cm (Fig. 1) and similar smaller lesions on the buttocks and left lower leg. Barium enema demonstrated a colon of normal appearance. Sigmoidoscopy was also normal and rectal biopsy showed no histological abnormality. Further investigations demonstrated biochemical and immunological changes typical of chronic active hepatitis and this diagnosis together with that of P.G. was made.

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The skin lesions were dressed three times daily with S.C.G. aqueous solution (2% W/V), administered in the form of Rynacrom nasal spray. The largest ulcer healed within 3 weeks leaving a pink scar (Fig. 2). The other lesions cleared simultaneously. At this point steroids and azathioprine were introduced and there has been progressive improvement in the patient's general condition and liver function tests over the ensuing months. Two months later however there was a recurrence of mild diarrhoea with occasional blood streaking of the stools; but again barium enema and rectal biopsy have been normal.

### *Patient 2*

An 87-year-old man, previously fit, was admitted with a large painful ulcerating lesion over the right lower leg which had developed during the previous month following a scratch. A diagnosis of infected leg ulcer was made and benzyl penicillin 1 mega unit 6 hourly together with lincomycin 600 mg 8 hourly was prescribed. Initially no pathogenic organisms were cultured from the lesion, but 2 weeks later a profuse growth of *Pseudomonas aeruginosa* was obtained on a number of occasions. The lesion progressed rapidly, eventually involving most of the circumference of the right lower leg (Fig. 3). At this time P.G. was diagnosed. Antibiotics were stopped and local treatment with S.C.G. was instituted as in Patient 1. There was immediate relief of pain and healing started within 2 days continuing thereafter (Fig. 4). Diarrhoea with faecal incontinence developed, however, and rectal and sigmoid biopsy showed minor inflammation of the submucosa, a barium enema revealing mild diverticular disease. Because of the possibility of underlying ulcerative colitis sulphasalazine in a dose of 500 mg 6 hourly was started 5 days later. The patient's general condition and the leg ulcer continued to improve until a month later when obstructive jaundice developed. This was at first thought to be due to sclerosing cholangitis, a complication of ulcerative colitis, and 33 days after commencing treatment with local S.C.G., oral steroids in a dosage of prednisone 40 mg daily were started because of his poor general condition. Five weeks later the real cause of the jaundice became apparent when he underwent successful laparotomy for a perforated gall bladder. The liver was seen to be normal and there were large gallstones. Sulphasalazine was stopped and steroids were phased out. At this point the post-operative course was satisfactory and his ulcer continued to heal. The healing of the ulcer started before the administration of either systemic steroids or sulphasalazine and continued after those compounds were withdrawn—being complete after a period of 3 months, when treatment with S.C.G. was stopped.

## DISCUSSION

Steroids and immunosuppressive drugs (Byrne, Hewitt & Summerly, 1976) have been used for P.G., agents which may also be appropriate for associated conditions such as ulcerative colitis, Crohn's disease and chronic active hepatitis. Clofazimine (Michaëlsson *et al.*, 1976), a drug which enhances granulocyte activity, cyproheptadine (Gelernt & Kreel, 1976), a platelet deaggregator, sulphasalazine (Perry & Brunsting, 1957) and local steroids (Gardner & Acker, 1972) have also been employed. Sometimes, however, and when associated with ulcerative colitis, the condition has reponded only to radical excision of the involved bowel.

We report two patients with P.G. who responded well to the local application of S.C.G. This compound has been used in the treatment of ulcerative colitis and proctitis (Heatley *et al.*, 1975) and it is suggested that it may act in these conditions by interfering with allergic mechanisms, in particular the release of histamine from sensitized mast cells. Certainly this is a known property of the compound and it is believed that it exerts its therapeutic effect in bronchial asthma in this way.

S.C.G. has not been found to be effective in allergic skin conditions and a dissociation between its cutaneous and respiratory activities has been noted (Assem & Monar, 1970; Patterson, Talbot &

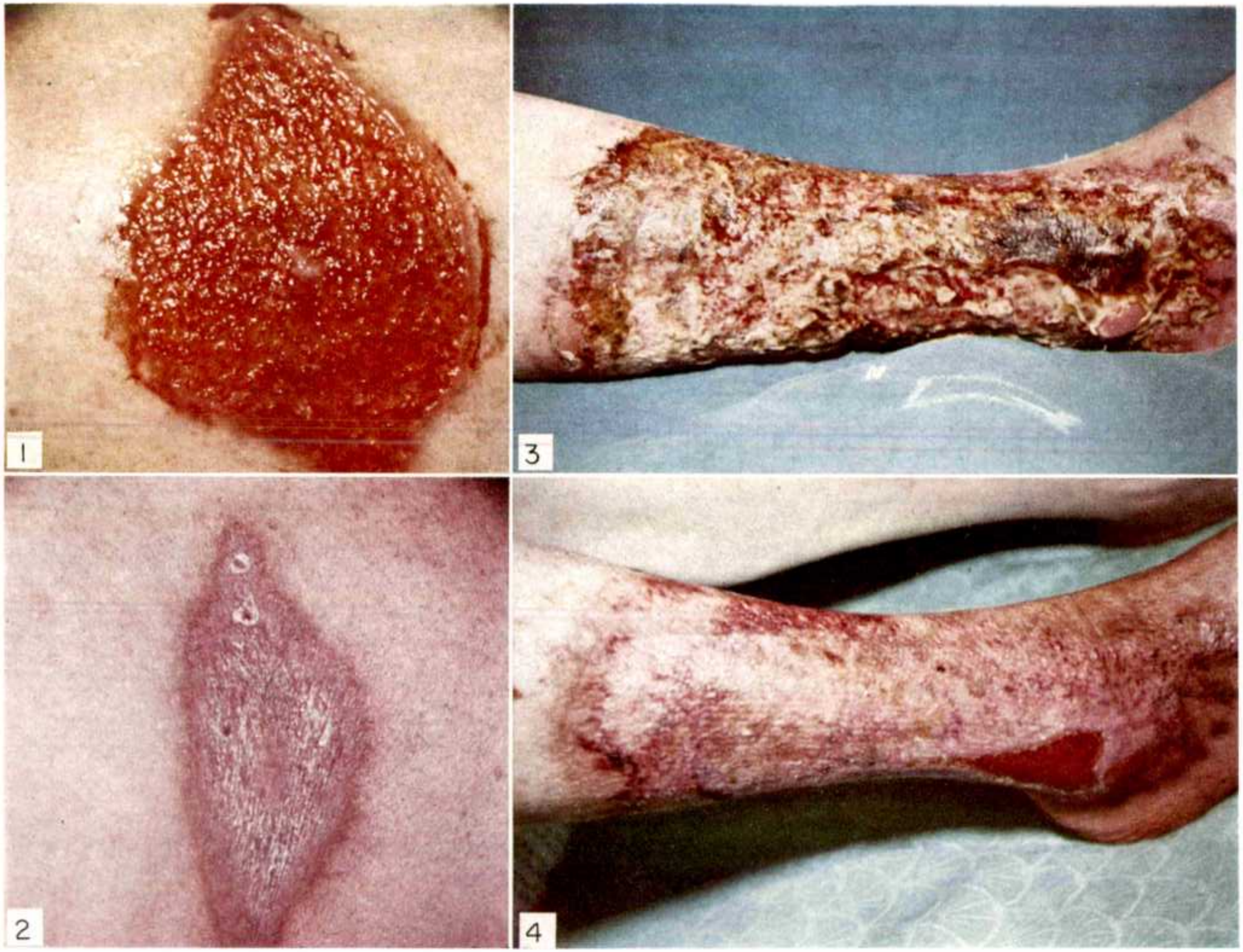

FIGURE 1. Case 1. Pyoderma gangrenosum on left thigh before treatment.
FIGURE 2. Case 1. Same lesion as in Fig. 1, after 6 weeks treatment with Rynacrom solution.
FIGURE 3. Case 2. Pyoderma gangrenosum on right lower leg before treatment.
FIGURE 4. Case 2. Same lesion as in Fig. 3, after 11 weeks treatment with Rynacrom solution.

Brandfonbrenor, 1971). The skin of patients with P.G. however has been shown to exhibit abnormal immunological responses (Lazarus *et al.*, 1972), and Rostenberg (1953), in his review of the Schwartzman reaction, emphasized the importance of the degranulation of mast cells in that situation and cited the strong resemblance between the Schwartzman reaction and P.G. An additional consideration is that S.C.G. may under certain circumstances inhibit type III (Arthus) reactions which are themselves capable of causing tissue damage in the lungs (Pepys *et al.*, 1968).

Finally it should be noted that the apparent beneficial effects of S.C.G. in P.G. may not be specific to that condition. Recently, for example, Lip (1978) has reported the healing of leg ulcers of unspecified type with this compound. In any case we feel that locally applied S.C.G. merits further trial in pyoderma gangrenosum.

## ACKNOWLEDGMENTS

We are grateful to Dr G.W.Manley and Dr A.Bowyer for the helpful interest they have shown in the preparation of this paper.

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*British Journal of Dermatology* (1980) **102**, 235.

## *Comment*

# PYODERMA GANGRENOSUM: A REAPPRAISAL OF ASSOCIATED SYSTEMIC DISEASES

Pyoderma gangrenosum is a unique cutaneous ulceration defined clinically by a raised, purple, tender, undermined border and an irregular, purulent base. Histological findings are non-specific, and include necrosis, epidermal ulceration, oedema, and infiltration by polymorphonuclear leukocytes and lymphocytes; vasculitis is usually absent. Pyoderma gangrenosum has been classically associated with ulcerative colitis, but our recent reappraisal suggests that this association is not as common as was formerly suggested (Hickman & Lazarus, 1979).

In the original classical description of pyoderma gangrenosum, Brunsting, Goeckerman & O'Leary (1930) noted that four of their five patients had ulcerative colitis. Reports appearing soon after the initial description of pyoderma gangrenosum were confined almost exclusively to patients with ulcerative colitis; indeed, pyoderma gangrenosum was considered a specific cutaneous complication of ulcerative colitis. Perry & Brunsting (1957) observed that pyoderma gangrenosum was not invariably associated with bowel disease, since only eleven of nineteen patients in their series had ulcerative colitis. Later, Perry (1969) reported that thirty-one of his sixty-two patients with pyoderma gangrenosum had ulcerative colitis. Consequently, the general impression in the literature has been that 50% of patients with pyoderma gangrenosum have ulcerative colitis. This figure may significantly overestimate the prevalence of ulcerative colitis in pyoderma gangrenosum.

Regional enteritis or Crohn's disease was reported in association with pyoderma gangrenosum in one patient by Van Patter *et al.* (1954). McCallum & Kinmont (1968), reviewing the dermatological manifestations of Crohn's disease, noted that pyoderma gangrenosum occurred infrequently. A recent review by Greenstein, Janowitz & Sachar (1976) described six cases of pyoderma gangrenosum in a series of 498 patients with Crohn's disease or regional enteritis.

A variety of other systemic diseases have been reported in association with pyoderma gangrenosum. Two patients with active chronic hepatitis and pyoderma gangrenosum were reported by Byrne, Hewitt & Summerly (1976). Rheumatological conditions including sero-positive rheumatoid arthritis, sero-negative symmetrical small joint polyarthritis, lupus erythematosus and the acute, oligoarticular sero-negative arthritis of ulcerative colitis are undoubtedly related to pyoderma gangrenosum (Lazarus *et al.*, 1972). Some patients with pyoderma gangrenosum have alterations in delayed hypersensitivity, paraproteinaemias and multiple myeloma.

A clinically distinctive more superficial bullous variety of pyoderma gangrenosum may occur with haematological malignancies, including blast crisis of polycythaemia vera or myeloid metaplasia, acute or chronic myelogenous leukaemia, and acute lymphoblastic leukaemia (Perry & Winkelmann, 1972).

It has become increasingly apparent that pyoderma gangrenosum is not limited to one systemic disease setting. Furthermore, it may appear without associated systemic disease. Review of patients with pyoderma gangrenosum treated at Duke University Medical Center (Durham, North Carolina) and North Carolina Memorial Hospital (Chapel Hill, North Carolina) from 1973 to 1977 revealed evidence of associated clinical disease in only seven of fifteen patients (Table 1). Twenty per cent of

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TABLE 1. Clinical disease associated with pyoderma gangrenosum

| Condition associated with pyoderma gangrenosum | | | Patients | |
|---|---|---|---|---|
| | | | No. | % |
| No associated disease | | | 8 | 53 |
| Gastro-intestinal disease | | | 4 | 27 |
| Ulcerative colitis | 1 | | | |
| Crohn's Disease | 3 | | | |
| Colitis | | 1 | | |
| Ileocolitis | | 1 | | |
| Regional enteritis | | 1 | | |
| Arthritis | | | | |
| Sero-positive rheumatoid arthritis | | | 3 | 20 |
| Total | | | 15 | |

the patients had Crohn's disease and twenty per cent had sero-positive rheumatoid arthritis. Only one of the fifteen patients had ulcerative colitis. The apparent prevalence of ulcerative colitis or any specific condition in pyoderma gangrenosum will vary according to the referral patterns to the reporting institution. However, several conclusions can be reached from our review: first, there is a significant incidence of pyoderma gangrenosum in patients with Crohn's disease. Second, appropriate evaluation of a patient with pyoderma gangrenosum should include not only a search for inflammatory bowel disease, but also studies to detect hepatic, rheumatological, immunological or hematological disease. Third, following such thorough evaluation, the dermatologist should feel justified in diagnosing pyoderma gangrenosum as a specific clinical entity without requiring underlying systemic disease.

The aetiology of pyoderma gangrenosum remains unknown. There is little data to support the notion that this syndrome is an example of the Schwartzman phenomenon. The diffuse elevations in immunoglobulin levels are not specific; the occasional dysproteinaemias are too uncommon to be a unifying mechanism. Depression of delayed hypersensitivity is common, but our recent studies indicate that it is not universal (Hickman & Lazarus, 1979). Alterations in polymorphonuclear leukocyte migration have also been reported, but this abnormality is also variable. We favour the hypothesis that pyoderma gangrenosum is an expression of altered immunological reactivity, but the precise defect has yet to be determined.

Therapy for pyoderma gangrenosum requires control of related underlying diseases. Good topical ulcer care is essential for healing. Often conservative management fails and corticosteroids injected into the lesion or administered systemically are necessary for resolution. Dapsone, azathioprine and cyclophosphamide are also effective, in our experience, as adjuncts to corticosteroid therapy. We have no experience with clofazamine but this agent has been reported to be effective in pyoderma gangrenosum (Michaëlsson *et al.*, 1976). The patient should be managed with great care. Abrupt alterations in drug regimens should be avoided since these may induce severe exacerbations of the ulcers. The prognosis for patients with pyoderma gangrenosum is usually good, but the clinician should always remember that this is a serious problem with a significant mortality.

## ACKNOWLEDGMENTS

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## Sweet's syndrome, pyoderma gangrenosum and acute leukaemia

SIR, The association of acute leukaemia with acute febrile neutrophilic dermatosis (Sweet's syndrome) appears to be well-recognized (Matta *et al.*, 1973; Klock & Oken, 1976; Pirard & Delannoy, 1977; Goodfellow & Calvert, 1979), and acute leukaemia has also been reported in association with pyoderma gangrenosum (Perry & Winkelmann, 1972; Crow & Bowers, 1974; Burton, 1976).

Although the classical features of Sweet's syndrome and pyoderma gangrenosum are fairly distinctive, the two diseases have some features in common, and some cases are difficult to categorize with certainty. A review of the cases associated with acute leukaemia (Pye & Choudury, 1977) has shown that some of them are not typical of either Sweet's syndrome or pyoderma gangrenosum (e.g. because of frank blisters and lesions of the oral mucosa). This diagnostic dilemma suggests either that Sweet's syndrome and pyoderma gangrenosum represent the two ends of a nosological continuum, or that acute leukaemia is associated with a different bullous pyoderma which may on occasion mimic either Sweet's syndrome or pyoderma gangrenosum. All authors who have reported such cases agree that the dermal cellular infiltrate is not composed of immature cells, and in some cases skin lesions appear in the aleukaemic phase, but further study of the infiltrate using immuno-cytological and electron microscopic techniques is required to confirm that the infiltrate is not malignant. Such studies may also yield useful information in typical cases of Sweet's syndrome and pyoderma gangrenosum which are not associated with leukaemia.

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## Perioral dermatitis

SIR, I write to support the conclusion of Dr Cotterill (1979) that perioral dermatitis can be caused by the use of steroids of all strengths greater than hydrocortisone. I have certainly seen it follow the use of hydrocortisone butyrate (Locoid) and clobetasone butyrate (Eumovate) on mild eczema.

At the same time I would protest that in my trial of hydrocortisone butyrate (Sneddon, 1973) I was attempting to assess whether in cases showing atrophy the hydrocortisone butyrate would prevent oxytetracycline controlling the eruption and it did not. I then made the incorrect assumption that as hydrocortisone butyrate did not interfere with the control of perioral dermatitis by oxytetracycline that it was less likely to cause it. In that I was wrong but I have never advocated its use alone as a primary treatment for perioral dermatitis. Misleading advertisements have implied this. In a later paper on the treatment of steroid induced rosacea and perioral dermatitis (Sneddon, 1976) I did say that 'the sooner the patient can be weaned off all steroids if suffering from rosacea or perioral dermatitis the better'.

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## Treatment of chronic urticaria

SIR, It was with interest that I read of the report of Commens & Greaves (1978) and then more recently Cook & Shuster (1979) of the lack of effect of $H_2$ blockade in the treatment of chronic urticaria.

I have recently completed a study along exactly the same lines as those described in the above mentioned papers. Twenty-two patients with chronic idiopathic urticaria of duration 3 months to 25 years (mean 36 months) completed a double-blind crossover trial comparing the effect of a placebo to chlorpheniramine (16 mg/day) alone and to a combination of chlorpheniramine (16 mg/day) plus cimetidine (1600 mg/day). Two patients were excluded from the analysis of results due to irregular tablet intake.

Results showed a significant improvement in the urticaria whilst on the chlorpheniramine alone or whilst on the combination of chlorpheniramine plus cimetidine when compared to the placebo. Analysis showed that there was no significant difference in improvement between the patients when they were taking the combination of chlorpheniramine plus cimetidine compared to when they were taking the chlorpheniramine alone.

These results agree with the statement by Cook & Shuster (1979) that support is at present lacking for the use of cimetidine in chronic idiopathic urticaria when used according to the study described.

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## Treatment of Hutchinson's melanotic freckle

SIR, Dawber & Wilkinson (*British Journal of Dermatology*, 1979, **101**, 47) are to be congratulated for contributing to the treatment of Hutchinson's melanotic freckle. If no clinical suspicion of invasiveness exists, simple modes of therapy are recommended, since most lesions occur on the face of the elderly. However, I do not support their contention that cryotherapy might be the treatment of choice for the invasive phase (lentigo maligna melanoma, LMM). My concern and criticism involves the following:

(1) I object to the definition 'nodular' to indicate the invasive stage of lentigo maligna. The nomenclature 'nodular melanoma' should be restricted to the corresponding histogenetic type of melanoma as proposed by Clark *et al.* (1969).

(2) The authors state that LMM was histologically confirmed. Probably punch or incisional biopsies were performed. The risk of dissemination of tumour cells due to incisional procedures should not be bagatellized. Van Slooten & Van der Esch (1977) reported on a significant reduction in survival of melanoma patients who had incisional biopsies prior to definitive surgery as compared to patients with radical surgery as the first treatment approach. In our institution the same trend was noticed: 5-year survival of fourteen patients with incisions through their melanomas amounted to 57%, versus 74% for sixty-two patients with primary radical treatment. Multivariate statistical analysis revealed this difference to be highly significant ($P = 0{\cdot}006$).

(3) LMM is a metastasizing tumour with a relatively slow growth rate. The follow-up of the patients of Dawber & Wilkinson is much too short (6–24 months) to draw meaningful conclusions about the bearing of conservative measures upon long-term survival.

(4) Not all melanomas on the face of the elderly patient are of the LMM-type. I fear that the encouragement of unconventional methods of therapy for LMM will result in inadequate treatment for the more malignant types of melanoma as well. Moreover, to distinguish between the various histogenetic types and to make an accurate assessment of the microstages according to Clark *et al.* (1969) and Breslow (1970), excision of the entire lesion seems mandatory whenever feasible (Roses *et al.*, 1979).

(5) The chronological age of the patient should never be a justification for limited or inappropriate treatment approaches, particularly not in oncological cases.

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SIR, The comments of Dr Rampen are to be welcomed since they represent, in principle, the prevalent view that Hutchinson's lentigo maligna melanoma (LMM) should receive essentially the same therapeutic considerations as other types of malignant melanoma. We consider this to be unsound on historical, clinical, histological and prognostic grounds. It is axiomatic that correct diagnosis be made prior to cryosurgery, or any other treatment; evidently in this study only classical, early nodular LMM lesions developing in chronic lentigo maligna have been treated (see Fig. 1 in our paper).

We cannot reiterate too strongly that the science of cryobiology has now given us a treatment that, when used correctly, is as destructive to cells as X-irradiation—and the surgeon's knife!

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*British Journal of Dermatology* (1980) **102**, 242.

# Book Reviews

**Dermatology in General Medicine,** 2nd edn. Edited by T.B.FITZPATRICK, A.Z.EISEN, K.WOLFF, I.M.FREEDBERG and K.F.AUSTEN (1979) New York: McGraw-Hill. Pp. 1884. Price £47.60.

This second edition of 'Fitzpatrick' is a splendid book; a monument to the industry and intelligence of its 174 eminent authors and its five august editors.

The book is slightly shorter than before, despite the addition of much new work, and this has been achieved by judicious pruning. A single volume textbook cannot be completely comprehensive, and though some of the 'very small print' conditions are missing, all the medium-rare diseases that I looked for were well covered.

As one would expect, the articles are uniformly authoritative and up-to-date. There are many references dated 1977 and a few for 1978, which is as good as one could hope for with so many contributors to be whipped towards the deadline. Though I searched hard, I found no statements with which I could categorically disagree. There were, of course, some opinions with which one might differ, such as the implication that the bullous pyoderma which occurs rarely in the aleukaemic phase of acute leukaemia is always due to the infiltration of malignant cells rather than to Sweet's disease or pyoderma gangrenosum, but these were isolated quibbles. More often, I checked up on some little-known entity about which I felt reasonably well-informed and found interesting references of which I had previously been unaware.

This latter observation may partly reflect a mutual lack of eclecticism in the reading of the literature. Although the number of European authors has been increased in this edition, there still remains an understandable American bias in the references selected. This detracts little from the book, except in the case of a few topics such as antiandrogen therapy. Ebling, in his section on the physiology of the hair-follicle, points out that antiandrogens decrease body hair, but in the clinical sections on acne and hirsutism, antiandrogen therapy is not mentioned even as a future possibility, despite the successful use of cyproterone acetate for these two conditions in thousands of European women in the last 10 years.

The overall production of the book is good and the editing is of a high standard, though confusion reigns with regard to the importance of psychosomatic factors in alopecia areata. We are told on p. 402 that these factors are of dubious significance, whereas on p. 1358 their importance is emphasized, even to the extent of specifying the 2-week interval to be expected between the psychological trauma and the onset of the hair fall.

A notable feature is the grouping together of more than seventy pages of colour pictures at the end of the book. The cost of these has been subsidised by eight listed drug firms and there is surely much scope for this type of collaboration in the future. The colour reproduction of some of these pictures is far from perfect however. The cafe-au-lait macule, which was appropriately pale brown in the first edition, now appears purple, and the horripilating lilac lips of the lady with rosacea have not responded to treatment in the intervening 8 years either.

My overall verdict is that this impressive tome represents excellent value for money. Its appearance is well timed and I'm sure every dermatologist will want his own copy.

Rook *et al.*, the ball is in your court, and it's a fizzer!

J.L.BURTON

**Dermatology Update: Reviews for Physicians.** Edited by SAMUEL L.Moschella (1979) New York: Elsevier. Pp. 456. Price $24.95.

This praiseworthy New World volume is in the image of our own popular half-century old series, *Recent Advances*. It is magnificently produced, authoritatively written by specialists respected in this country (many of them friends to boot!), adequately modern alphabetically listed references mostly from this decade and originating from both shores of the Atlantic, evenly edited, and fairly priced; in essence a rewarding 'weekend read'. It differs favourably from our *Recent Advances* editions in having clinical photographs (although I deplore prospective authors not anticipating their needs by ordering black and white rather than attempting wishy-washy transmogrifications of their colour snaps). The book thereby approximates more to a standard teaching text. But is it more than that?

The words of the Editor-in-Chief's preface 'a variety of subjects are reviewed in depth . . . current and complete references . . . significant progress is reported . . .' and so forth are gloriously substantiated, and many of the chapters are thoroughly interesting. 'In depth' certainly applies to the chapters concerning pseudo-malignant and pseudo-benign skin lesions (fifty pages), heritable disorders of connective tissue, prenatal diagnosis of heritable skin diseases, and mixed connective tissue disease (and there are two chapters on the sclerodermas as well). I enjoyed the chapters that summarized large subjects selectively; malignant melanoma, systemic disease and light, T-cell lymphoma, and vitiligo and its relationship to systemic disease. A wide choice of subjects certainly ensures that all tastes will be gratified. (Junior staff nonetheless must study every chapter!) Paediatric dermatology is threatening as an active splinter group, so the mentions of children's disorders in various chapters and the chapter entitled 'Life threatening diseases of childhood' are opportune. The speculations in both 'Neutrophilic dermatoses' and 'Pyoderma gangrenosum' intrigued me and clearly pointed to gaps that future investigators must block. Many will be pleased to be 'updated' on HLA, the bullous diseases, and angiitis (where I was pleased to read that it is now respectable to believe that the most common segment of the cutaneous vasculature to be hit by disease as seen by dermatologists is the venule and that the myth of 'arteriolitis' is being exorcised).

My task, is to help *confrères* embarrassed by the high quality of newly available literary dermatological riches to equate them with the lightness of their purses. All dermatologists, whether pupating or established, will have to own the third edition of 'Rook *et al.*' but is *Dermatology Update* sufficiently different or original to justify all of us buying a copy as well? I would never have been tempted to read elsewhere complete chapters on systemic mycoses, mycobacterial infections, and prenatal diagnosis of skin diseases, but their clear presentation in the opening three chapters of *Dermatology Update* enticed me, and I have benefited. All in all, I find little to criticise adversely, much to applaud, and I will now confess that the book has proved to be too much of a *bonne bouche* for a weekend and I shall be constrained to take it on train journeys and on holiday for many more hours of rumination and pleasure. I've packed it!

P.W.M.COPEMAN

**Leprosy,** 2nd edn. ANTHONY BRYCESON and ROY E. PFALTZGRAFF (1979) Edinburgh: Churchill Livingstone. Price £4.

The book is one of a series of handbooks published by Churchill Livingstone on Medicine in the Tropics, and in its first edition was called *Leprosy for Students of Medicine*. It is a sign of the times that it appears to be printed in Singapore.

Not long ago dermatologists would murmur amongst themselves that leprologists made their single disease totally incomprehensible because of a tangle of nomenclature, which varied from one exponent to another. A similar accusation could, of course, always be levelled at dermatologists. But the authors of this book have made triumphant sense of the disease which, happily, forms part of the work of dermatologists. The book is born of a course for medical students which is given at Ahmadu Bello University, Nigeria and he is a lucky man who is taught there.

The text is both practical and wide-ranging, placing the disease in a full background of modern scientific understanding. The sections on immunology, immunological complications, management of reactions, the eye in leprosy, and the recognition and treatment of dapsone-resistant leprosy are particularly good. Up-to-date references at the end of each chapter hardly apply to student teaching and will educate physicians at all stages of senility.

It is a pity there are no colour photographs — neither of clinical material nor of histology. Lepromatous leprosy (LL) histology is particularly disappointing seen in black and white, and Plate 4 seems not to be in focus. The arrangement of illustrations is sometimes bizarre, with a nine-banded armadillo (which abounds in the Louisiana swamps near Carville) standing on what seems to be a table next to a picture of gynaecomastia and Type 2 reaction.

Not all the annoying mistakes, which the authors were glad to see removed from the first edition, have been rectified. Page 43 tells us that the M.I.C. of dapsone in mice infected with *M. leprae* is 0·015 μg/ml, whereas page 122 gives the same value as mg/ml. But these are petty criticisms.

The book is to be highly recommended for every student and doctor with a feeling for this devastating disease. The fresh approach to teaching due to the combination of research, laboratory and clinical expertise of the authors gives new impetus to anyone who is already interested.

R.R.M.HARMAN

**Genetic Diseases of the Skin.** V.M.DER KALOUSTIAN and A.K.KURBAN (1979) Berlin: Springer-Verlag. Pp. xv+339, 441 B/W illustrations.

This new text on genetic diseases of the skin is a product of a very effective collaboration between a paediatrician and a dermatologist in Beirut, Lebanon. The authors have aimed at including every clinical entity which is certainly or probably hereditary. After a short introductory chapter on human genetics supported by an extensive bibliography, the hereditary disorders are systematically reviewed. There is a short account of the clinical features and where this is relevant, of treatment; neither the text nor the lists of references are intended to be exhaustive. One chapter is devoted to dermatoglyphics and one to cancer, genetics and the skin. A useful appendix tabulates the differential diagnosis of hereditary disorders on the basis of dermatological signs. The great majority of conditions covered are illustrated. Photographs have been most carefully selected and, although some of them are not of outstanding technical quality, they all effectively illustrate the particular feature they are intended to portray.

The brevity of the text makes a degree of dogmatism inevitable but there are remarkably few of the authors' statements to which it is possible to take exception. However the account of acrokeratoelastoidosis is confused. This term has been applied by different authors to at least two conditions. As Jung and his colleagues showed in 1974 one of these entities is a very distinctive autosomal dominant palmoplantar keratoderma, beginning in childhood. Another is a degenerative connective tissue change reported from Brazil by Costa and from Egypt by a number of authors.

Several new hereditary syndromes are discovered each year and some well established syndromes are proved to be heterogeneous. The authors of this extremely useful text are to be commended for the thoroughness of their survey of recent literature. This book will be invaluable for the dermatologist or paediatrician who wishes to improve his diagnostic range and accuracy.

ARTHUR ROOK

**Dermatologie Néo-Natale.** D. WALLACH (1979). Paris: Vigot. Pp. 141, 81 figures, many in colour, 7 tables.

This is a short, clearly presented account of neonatal dermatology. The author has skilfully maintained an appropriate balance between the common problems and the rarities most of which are included with just sufficient detail to enable the reader to consider them in differential diagnosis, and with a well selected list of references to the recent literature. The illustrations, about half of which are in colour, are small but most of them are effective; a few should be replaced in future editions.

In his preface Professor Rossier says that until now we have lacked an illustrated manual of neonatal dermatology. This is of course not so, and Dr Wallach himself refers to the work on this subject by Solomon and Esterly. We can however agree with Professor Rossier in congratulating Dr Wallach on his attractive and useful monograph. The experienced dermatologist and paediatrician will appreciate the carefully compiled tables of differential diagnosis and the excellent bibliographies which occupy over ten per cent of the book.

ARTHUR ROOK

*British Journal of Dermatology* (1980) **102**, 245.

# *News and Notices*

## Principles of Cosmetics for the Dermatologist: 1980

An international symposium on Principles of Cosmetics for the Dermatologist, sponsored by the Department of Dermatology, Mount Sinai Medical Center, Miami Beach, will be held at the Omni International Hotel, Miami, Florida, U.S.A. on 1–3 May 1980.

Further information may be obtained from: Dr Phillip Frost, Chairman, Department of Dermatology, Mount Sinai Medical Center, 4300 Alton Road, Miami Beach, Florida 33140, U.S.A.

## Appointment

### Plymouth Health District

Dr Michael Glanmor Davies has been appointed Consultant Dermatologist to the Plymouth Health District.

He was educated at Cheltenham College and received his pre-clinical and medical training at Bristol University. He qualified B.Sc. in Biochemistry (Hons.) in 1967 and M.B. Ch.B. in 1970. After 2 years in Bristol he continued his general medical training in Cardiff and obtained the M.R.C.P. in 1973.

His dermatological training began in 1974 at Cardiff and in 1976 he became an M.R.C. Training Fellow in Dermatology concerned with the extraction and quantitation of epidermal glycosaminoglycans. Since August 1978 he has been working as Senior Registrar at St John's Hospital for Diseases of the Skin, London. His major interest is in the wider nature of the gluten-induced skin disease—dermatitis herpetiformis.

# International Coding Index for Dermatology

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Compiled by
Suzanne Alexander and Alan B. Shrank

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1978. 112 pages. Paper, £2.00

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# Clinical and Experimental Dermatology

Edited by Martin M. Black
*Institute of Dermatology, St. John's Hospital for Diseases of the Skin, London, England*

This journal publishes original articles covering all aspects of the normal biology of the skin and clinical and experimental dermatology. Regular features include the full text of bi-annual orations by internationally renowned speakers on topics of major dermatological interest and Society clinical proceedings. Invited review articles, book reviews and correspondence are published occasionally. *Clinical and Experimental Dermatology* was formerly titled *Transactions of the St. John's Hospital Dermatological Society* and, although the official organ of the hospital, attracts contributions from all countries and its circulation is equally international.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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**Manuscripts** should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) $(10^{-3}$ g$)$ | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) $(10^{-6}$ g$)$ | μg | cubic millimetre(s) | $mm^3$ | micrometer(s) | μm |
| nanogram(s) $(10^{-9}$ g$)$ | ng | millimetre(s) | mm | per cent | % |
| picogram(s) $(10^{-12}$ g$)$ | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hour(s) | h | milliequivalent | mmol | | |

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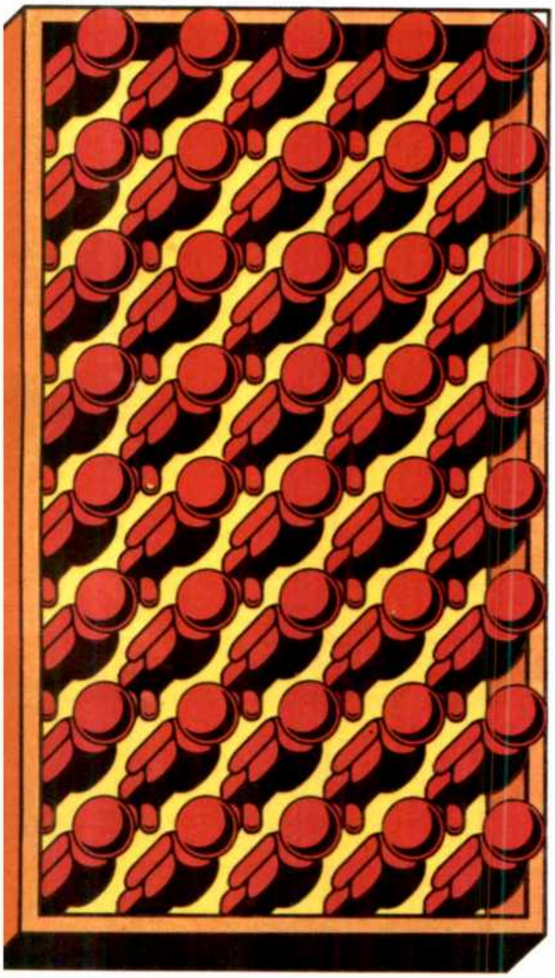

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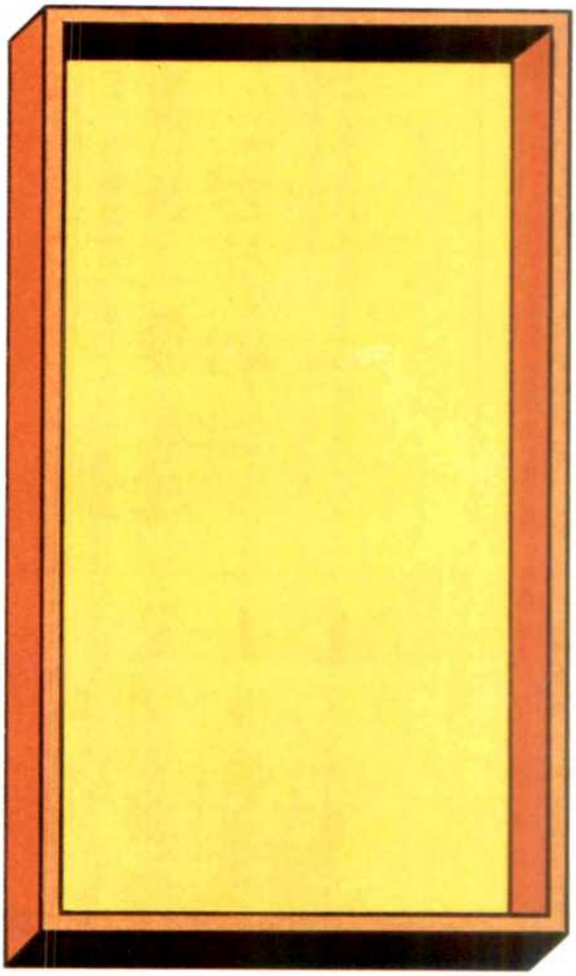

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**Contra-indications, warnings:** keep away from mouth, eyes and other mucous membranes and avoid normal skin. Irritant dermatitis reported in 2.5% to 3% of cases. If undue skin irritation develops discontinue product use. May bleach coloured fabrics. FOR EXTERNAL USE ONLY.

**Pack and NHS price:** bottles of 100ml, £3.00

PL 0174/0036

Canad. Med. Assoc. J. 1976, **115,** 1101 J. Dermatol. Surg. Oncol 1978 **4:** 1, 91 Cutis, 1978, *21,* 491 J. Dermatol. Surg. Oncol. 1978, **4:** 9, 701 Canad. Fam. Phys., 1976, **22,** 1301

Benoxyl is a registered trademark

Further information and Dressing Chart is available from

**Stiefel Laboratories (UK) Ltd**
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flamazine™
SILVER SULPHADIAZINE 1% w/w cream

**flamazine cream**
-for infected leg ulcers and pressure sores
Flamazine presents the broad spectrum antibacterial action of silver in a non sensitising and soothing cream.

Further information is available on request from:

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Telephone: WELWYN GARDEN (070 73) 25151

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W. Raab

**The Treatment of Mycosis with Imidazole Derivatives**

Foreword by A.M. Kligman
Translated from the German by T.C. Telger

1980. 41 figures, 19 tables. Approx. 180 pages.
DM 28,–; approx. US $15.70
ISBN 3-540-09800-3

In the face of rising fungal infections, the introduction of imidazole derivatives has been a highly welcomed addition to antimicrobial therapy. These compounds – which include clotrimazole, miconazole and its stereoisomer isoconazole, econazole and ketoconazole – are active not only against all types of fungi pathogenic to man, but also against gram positive bacteria. From the standpoint of the clinical pharmacologist, the use of these compounds for local treatment is almost ideal: indicated compounds are well tolerated, show almost no sensitization or photoreaction, and are easily absorbed.
This book summarizes the principles underlying the development of new antimicrobial agents. It provides much-needed information on antimycotics and antimicrobials in general, based primarily on the drug econyzole, and considers the question of whether antimicrobials should be combined with glucocorticoids in topical therapy. In particular, the practicing physician is shown the therapeutic results that can be expected from the topical treatment of mycotic infections with imidazole derivatives under ideal conditions.

**Contents:** Introduction.– Broad-Spectrum Antimicrobials for Local Application.– Econazole.– General Microbiology of Imidazole Derivatives for Local Application.– Therapeutic Use of Imidazole Derivatives in Animals (Experimental Therapy).– General Pharmacology of the Imidazole Derivatives in Man and Animals.– Toxicology of the Imidazole Derivatives.– Clinical Pharmacology of Topical Antimicrobials with Special Regard to the Imidazole Derivatives.– Microbial Infections in Man.– Mycoses.– Systemic Administration of Antimycotically Active Imidazole Derivatives in Man.– Local Application of Antimycotically Active Imidazole Derivatives in Man.– Combined Use of Imidazole Derivatives and Glucocorticoids for the Local Treatment of Skin Diseases.– Assessment of Mycoses in Various Branches of Medicine.– Concluding Remarks.– References.– Subject Index.

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Berlin Heidelberg New York

1516/4/2q

# Difficult Dermatoses Dermovate

**(clobetasol propionate)**

Even long-standing, stubborn eczematous lesions respond quickly to Dermovate – with rapid relief from itching; and this is often followed by prolonged periods of remission. And because Dermovate is so rapidly effective, treatment need be of short duration only, minimising the risk of steroid side effects.

**Prescribing information**

**Dosage and administration**
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.
**Side effects**
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.
This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.
**Precautions**
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.
Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.
**Contra-indications**
Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines.
Viral diseases of the skin, e.g., herpes simplex.

**Basic NHS cost (exclusive of VAT)**
Dermovate Cream or Ointment 25 gram tubes £1·74 (also available in 100 gram tubes).

| **Presentation** | **Product Licence number** |
|---|---|
| Dermovate Cream | 4/0219 |
| Ointment | 4/0220 |

***Glaxo***
**Leaders in topical steroid therapy**
Further information on Dermovate (trade mark) is available from:
Glaxo Laboratories Ltd
Greenford, Middlesex UB6 0HE

# People with psoriasis tend to hide themselves

It's not just the unsightly lesions. It's also the sheer discouragement, even despair. The available treatments seem to be either inadequate or cosmetically repugnant.

Alphosyl HC gives the psoriatic – and her doctor – grounds for hope. It is a cosmetically acceptable cream containing coal tar to slow down the psoriatic process, hydrocortisone in low dosage for a controlled anti-inflammatory effect, and allantoin to soften psoriatic plaques and aid in their removal.

A published clinical study* has shown Alphosyl HC to be broadly comparable in efficacy to betamethasone, and somewhat superior in its descaling action. And Alphosyl HC is preferable on safety grounds since it does not contain a fluorinated steroid.

# Alphosyl HC

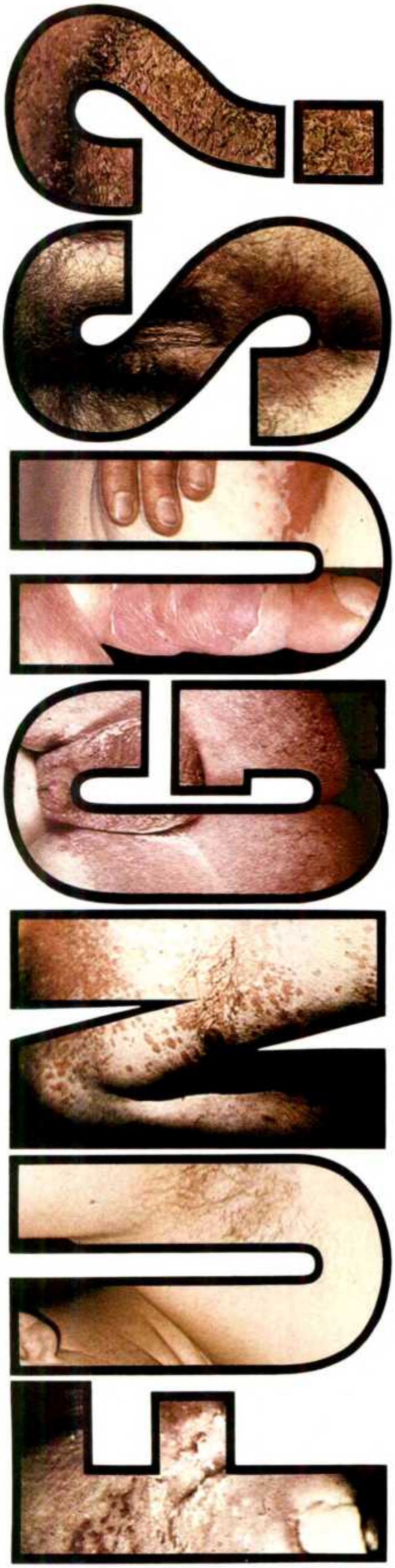
hydrocortisone 0.5% with allantoin and coal tar extract.

**purpose-designed for psoriasis**

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol. 33 No. 4, 106.

**Presentation** A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, u.s.p. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and Administration** Apply two to four times daily massaging vigorously into the affected areas. **Contra-indications and Warnings, etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tubes of 30gm and 45gm. **Basic NHS cost** £1.62 for 30gm, £2.29 for 45gm. **Product Licence No.** 0036/0026

Further information is available from
**Stafford-Miller Limited**, Professional Relations Division, Hatfield, Herts. AL10 0NZ



# Canesten®

clotrimazole

## Effective against all fungal skin infections

So effective treatment with Canesten may begin at once – without waiting for culture results.

Wherever on the body a fungal infection occurs, whatever the organism responsible, there is a need for speedy effective therapy. Canesten's broad range of presentations is the answer... cream, atomiser spray and now Canesten powder for adjunctive therapy and to help prevent re-infection.

## Canesten

the most widely-prescribed antifungal

**Prescribing Information**

**Presentations** *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder containing 1.0% clotrimazole.

**Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections.

**Dosage and Administration** *Cream, Atomiser Spray* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection.

**Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation.

**Precautions** Canesten atomiser spray should not be used near a naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled.

**Product Licence Numbers** Cream: PL 0010/0016. Atomiser Spray: PL 0010/0060. Powder: PL 0010/0067.

**Basic NHS prices** Cream: 20g, £1.37; 50g, £3.20. Atomiser Spray: 40ml, £3.68. Powder: 30g, £0.99.

Further information is available from
Bayer UK Limited, Pharmaceutical Division,
Haywards Heath, West Sussex RH16 1TP.
Telephone Haywards Heath (0444) 57911.

Distributed in the Republic of Ireland by ·
Bayer (Ireland) Limited, Dun Laoghaire, County Dublin.

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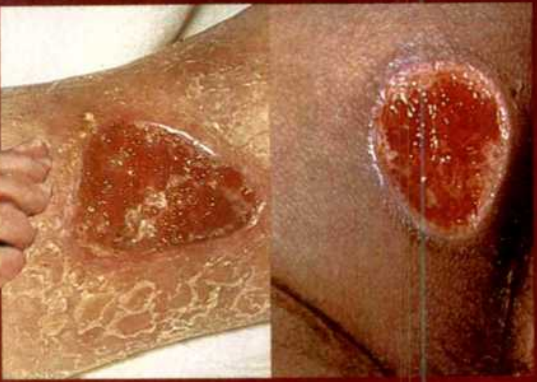
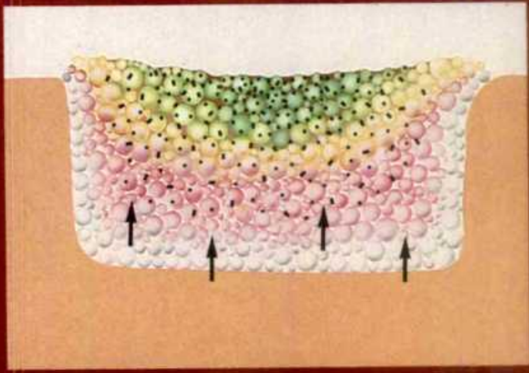
**Presentation** Sterile, straw-coloured spherical beads of dextranomer of 0.1-0.3 mm diameter, packed in plastic castors or single-use sachets.
**Uses** A dressing for moist, small area burns, wounds and indolent ulcers, whether clean or infected.
**Dosage and administration** A 3 mm layer of Debrisan should be sprinkled on to the wound and kept in place by a pad of lint or a perforated plastic sheet. Debrisan is hydrophilic and the tissue exudate is drawn up into the layer. The Debrisan should be renewed before saturation occurs. Depending on the rate of exudation this may be necessary from one to five times a day. Twice daily is usually adequate. When Debrisan is changed the old material is readily rinsed off with water or saline and new material may then be sprinkled on to the wound. Full instructions for use are enclosed with each pack. Treated in this manner the wound will remain soft and pliable during healing.
**Contra-indications, warnings, etc.**
1. Do not leave Debrisan for more than 24 hours on wounds with a very low exudation rate as it may dry and form a crust which may be difficult to wash off.
2. Occlusive dressings may lead to maceration of skin round the wound under treatment.

**Where** On all secreting wounds such as leg ulcers, pressure sores, post-surgical infections, Debrisan actively speeds healing.



**When** As soon as such wounds present, apply Debrisan and renew as necessary. 'The quality as well as the rapidity of wound healing has been impressive.'[1]

**How** By capillary action Debrisan continuously removes wound exudate, with bacteria and fibrinogen drawn up into the top layer. Inflammatory mediators are removed and painful oedema then subsides. Local blood flow should improve and the wound base is rendered clean and granulating.



# Debrisan

**dextranomer**

Rx

# DEBRISAN

## Unique fast-acting wound therapy

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[1] Thorne N. (1979) Brit. J. clin. Pract. **33**, 21

3. When deep infected wounds are treated, care must be taken to wash Debrisan from the depths of the wound.
4. No side-effects have been reported.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Precautions:

1. Not to be used on dry wounds.
2. When exudate has been markedly removed by Debrisan alternative treatment should be substituted.
3. In order to avoid cross-infection it is recommended that the contents of a castor be confined to the treatment of a single patient for one day.

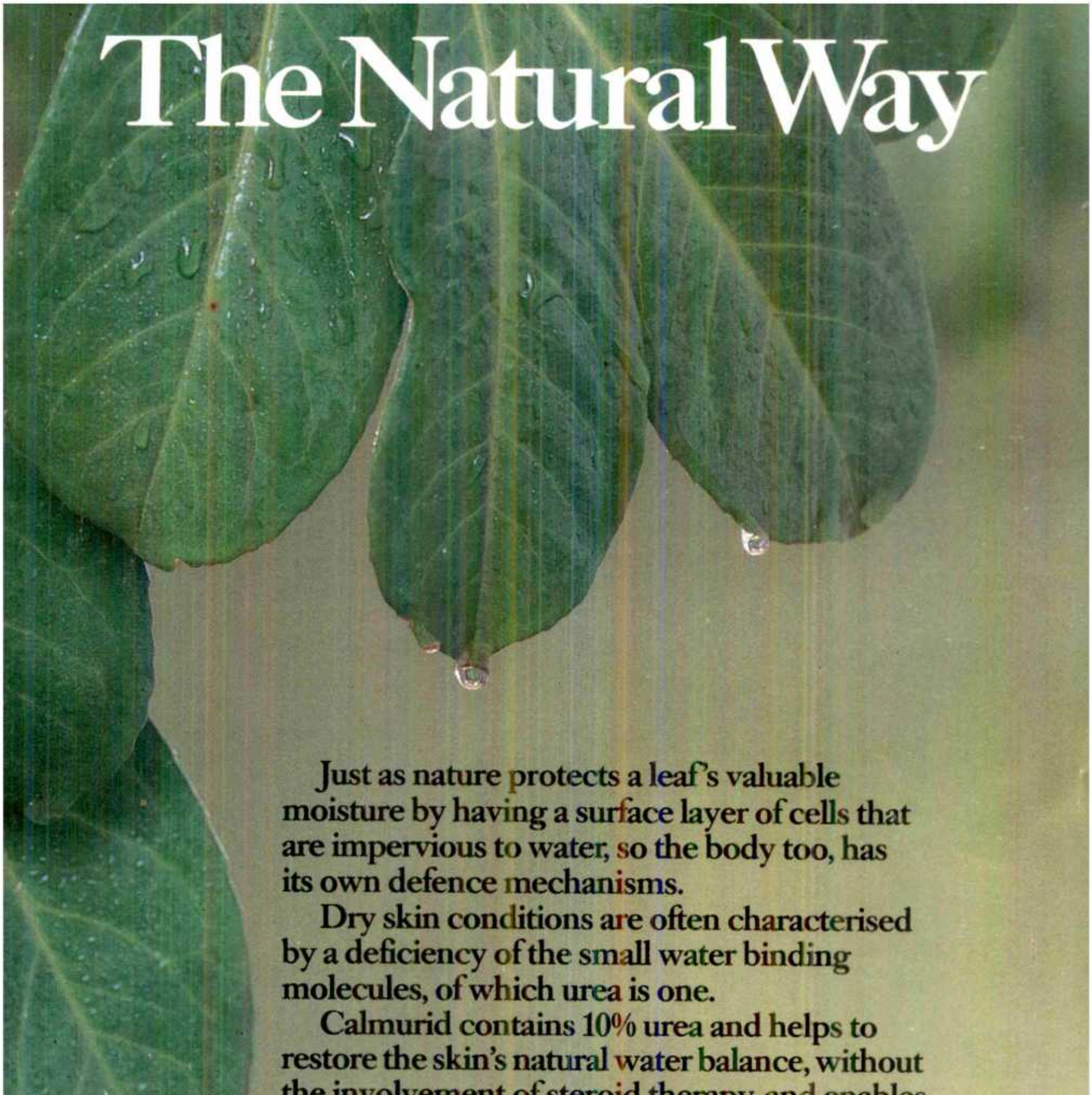
**Pharmaceutical precautions** Keep in a dry place in well-closed containers.

**Package quantities** Sachets of 4 g in boxes of seven. Basic NHS price £1.60 each. Castors of 60 g.

**Legal category** POM. **PL** 0009/0021

Debrisan (regd) dextranomer is a product of Pharmacia (Great Britain) Ltd, Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321
Further information is available on request to the Company.

# The Natural Way



Just as nature protects a leaf's valuable moisture by having a surface layer of cells that are impervious to water, so the body too, has its own defence mechanisms.

Dry skin conditions are often characterised by a deficiency of the small water binding molecules, of which urea is one.

Calmurid contains 10% urea and helps to restore the skin's natural water balance, without the involvement of steroid therapy, and enables it to return to its normal supple texture.

# Calmurid
urea
## helps the skin regain its moisture

**Prescribing Information**
**Presentation.** A white shiny cream containing Carbamide (urea) B.P. 10% in a stabilising emulsified base.
**Uses.** Treatment of dry hyperkeratotic skin conditions.
**Dosage and Administration.** After washing the affected skin apply a thick layer and leave 3-5 minutes. Apply twice daily, reducing dosage with improvement. Hyperkeratotic feet: soak in warm water, dry with rough towel and apply as above.
**Contra-indications, warnings etc.** Smarting may occur on fissured or sensitive skin.
**Pharmaceutical Precautions.** Store in a cool place.
**Legal Category.** G.S.L.
**Package Quantities.** Tubes of 50g, 100g and 300g.
**Further Information.** Lanolin, preservative and steroid free. PL 0009/5009.
50g Calmurid Cream £1.25 Basic N.H.S. price.

**Pharmacia**

Calmurid (regd.) urea B.P. 10% cream is a product of Pharmacia (Great Britain) Ltd., Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321
Further information is available on request to the Company.

# When efficacy is the need and safety the concern

No need now to sacrifice efficacy for safety because Eumovate fulfils the need for a corticosteroid preparation with greater topical activity than hydrocortisone, yet with a wide margin of safety.

# Eumovate

(clobetasone butyrate)

**a unique balance of efficacy and safety**

**Prescribing information**

**Uses**

Eumovate is suitable for treating the
forms of eczema, seborrhoeic dermat
and other steroid responsive skin
conditions.

**Dosage and administration**

Apply up to four times a day until
improvement occurs, when the frequ
of application may be reduced.

**Contra-indications**

Bacterial, fungal or viral diseases of t
skin.

**Precautions**

In infants and children, long-term
continuous topical corticosteroid the
should be avoided where possible, as
adrenal suppression can occur. In inf
the napkin may act as an occlusive
dressing, and increase absorption. T
least potent corticosteroid which will
control the disease should be selecte
Appropriate antimicrobial therapy sh
be used concurrently whenever treat
inflammatory lesions which have bec
infected.
Any spread of infection requires
withdrawal of topical corticosteroid
therapy and, possibly, commenceme
systemic chemotherapy.
As with all corticosteroids, prolonge
application to the face is undesirable
Topical steroids should not be used
extensively in pregnancy, i.e., in larg
amounts or for prolonged periods.

**Side effects**

In the unlikely event of signs of
hypersensitivity appearing, applicatio
should stop immediately. If large are
were to be treated, some patients co
absorb sufficient steroid to cause tra
adrenal depression despite the low d
of systemic activity associated with
clobetasone butyrate.
Local atrophic changes could occur
moisture increases absorption of ste
but only after prolonged use.

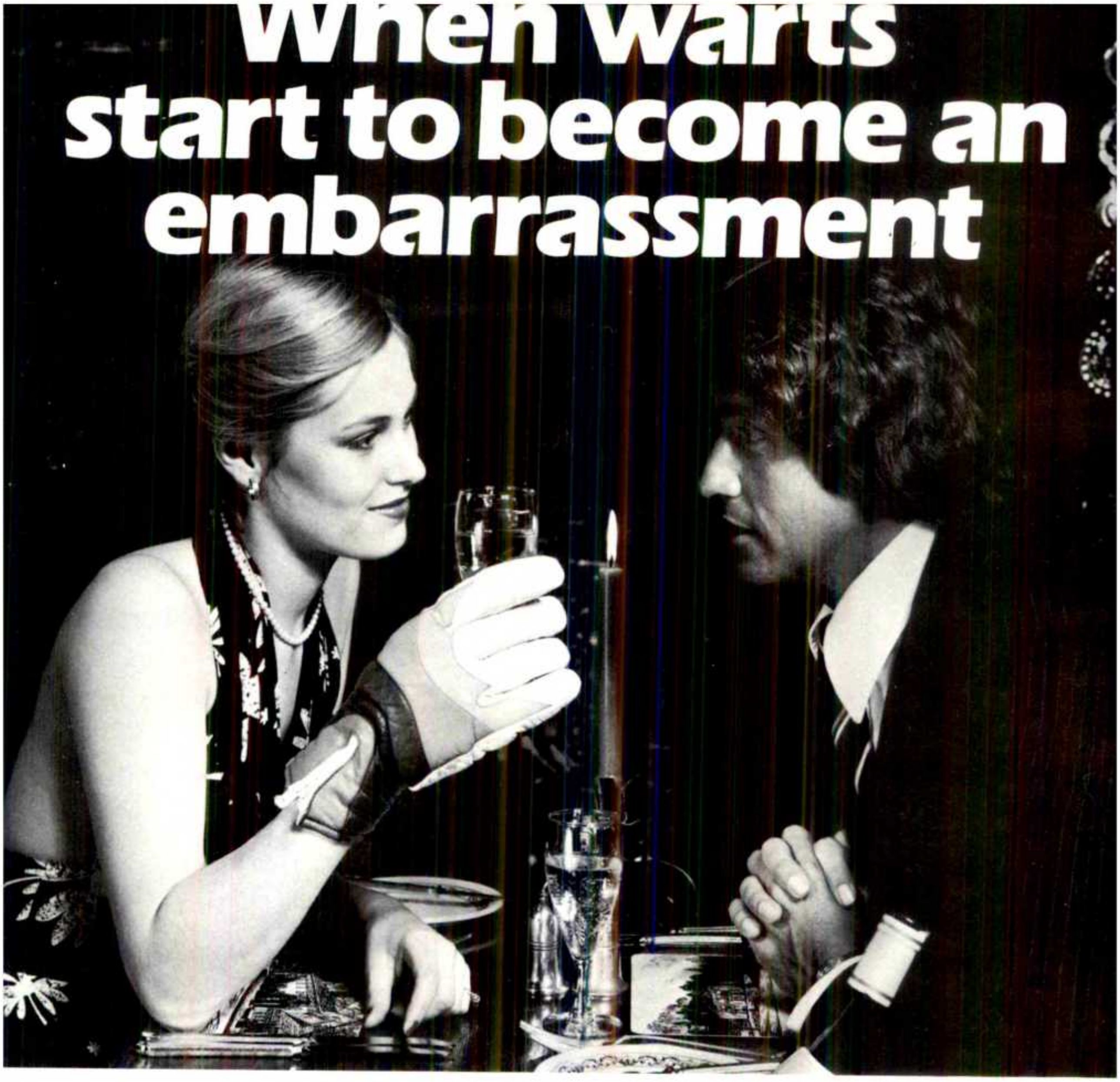
**Basic NHS cost** (exclusive of VAT)
25 gram tube £1·48 (also available in
100 gram tubes)

**Product Licence numbers**
Cream: 4/0233
Ointment: 4/0254

**Glaxo**

**leaders in topical steroid th**

Further information on Eumovate (tr
mark) is available from:
Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE



Many wart sufferers find their condition an embarrassment. Others – with plantar warts – suffer considerable pain on walking.

Both groups of patients will benefit from treatment with Salactol – the most widely prescribed wart preparation in the UK.

Salactol exerts a powerful keratolytic action to break up the wart tissue which then detaches from the surrounding skin.

In a comprehensive controlled clinical trial[1], Salactol was found to be more effective than other preparations. 84% of patients treated with Salactol were cured within twelve weeks.

Supplied complete with special spatula for ease of application, Salactol is particularly convenient for use by patients at home.

Full prescribing information is available from:
Dermal Laboratories Limited,
Gosmore, Hitchin, Hertfordshire SG4 7QR.

*Reference:* 1. Practitioner, **207**, 197, 1971

**Prescribing Information**
*Presentation:* An evaporative collodion paint containing 16.7% Lactic Acid BP, 16.7% Salicylic Acid BP and 66.6% Flexible Collodion BP.
*Indications:* For the treatment of warts, particularly plantar warts.
*Directions for Use:* Paint the wart, cover with a waterproof plaster and leave for 24 hours. Remove collodion by rubbing with a piece of pumice stone and repeat application.
*Contra-indications:* Warts around the eyes.
*Precautions:* Keep away from the eyes.
*Package quantities and Basic NHS price:* 10ml amber glass bottle fitted with applicator £0.98.
*Product licence No:* PL 0173/5006
Salactol is a registered trade mark.

**Salactol**

(lactic acid BP, salicylic acid BP)

*British Journal of Dermatology* (1980) **102**, 247.

## *Clinical and Laboratory Investigations*

# Immune reactions in epidermodysplasia verruciformis

S.PYRHÖNEN*, S.JABLONSKA†, S.OBAŁEK† AND E.KUISMANEN*

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Accepted for publication 22 May 1979

### SUMMARY

Serum antibodies against human papilloma virus have been measured using immunodiffusion and enzyme linked immunosorbent assay in twelve patients with epidermodysplasia verruciformis (EV). The results were compared with those from sixty-six patients with other types of human papilloma virus diseases. Antibody prevalences were slightly lower in patients with EV than in the control patients. More remarkable was the difference between EV patients and control subjects in tests of non-specific cell-mediated immunity, which was markedly depressed in the majority of patients with EV, but in the main preserved in patients with common warts. No specific immunological parameter was shown to be associated with malignant conversion of EV lesions, but malignancy was linked to the character of the inducing virus, and particularly to HPV type 5.

Epidermodysplasia verruciformis (EV) is a disease characterized by multiple wart-like skin lesions often distributed over the whole body. The disease usually starts during childhood and it is unique for its hereditary character and transformation into malignancy which in some reports has occurred in all patients (Rueda & Rodriguez, 1976) though in others in about one-third of them (Jablonska, Dabrowsky & Jakubowicz, 1972; zur Hausen, 1977). Typical human papilloma virus (HPV) particles have been observed repeatedly in lesions (Ruiter & van Mullem, 1966; Aaronson & Lutzner, 1967; Jablonska *et al.*, 1968; Yabe *et al.*, 1969; Delscluse *et al.*, 1972; Yabe & Sadakane, 1975; Bundino, Zina & Bernengo, 1978). Immunological factors, such as antibodies and cell-mediated reactions against the virus or some cellular components, are thought to confer protection and induce regression of warts (Pyrhönen & Johansson, 1975; Morison, 1975; Pyrhönen, 1978b). Impairment of these immunological mechanisms could be one reason for the development of such multiple warts and malignant changes as in EV disease. Recent studies on a small number of patients with EV have revealed some general impairment of cell-mediated immunity (CMI) (Glinski *et al.*, 1976; Dymock *et al.*, 1977; Prawer *et al.*, 1977; Bundino *et al.*, 1978). These observations may reflect either an inherited abnormality in immune functions or depressed immune functions due to the viral infection.

Apart from studies on general non-specific immune reactions in patients with EV, nothing is known

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of the specific immunity against the human papilloma virus. The present study was thus aimed at investigating the antibody response against HPV in patients with EV.

## PATIENTS AND METHODS

### *Patients*

The studies were carried out on twelve patients with epidermodysplasia verruciformis (Table 1), and as controls on nine patients with longstanding flat warts, on forty-two patients with common warts, on four patients with plantar warts and on eleven with condyloma acuminata (genital warts). Tests for non-specific cell mediated immunity (CMI) were performed on all the patients with EV and on a comparable series of fifty-nine patients with other types of HPV diseases, i.e. lymphocyte blast transformation to non-specific mitogens (phytohaemagglutinin and concanavalin A), evaluation of the percentage of E-rosette forming lymphocytes (Eibler & Morton, 1968; Wybran, Cabr & Fudenberg, 1972; Zeylemaker *et al.*, 1974), and quantitative response to DNCB sensitization (Glinski *et al.*, 1976). It should, however, be noted that not infrequently there are some divergent results with the various *in vitro* tests for CMI. The most reliable index of CMI seems to be the sensitization to DNCB. A negative response to the highest provoking dose of 100 μg is a sign of anergy. The following criteria for CMI tests were used: CMI depressed—negative response to 100 μg DNCB and at least one of the *in vitro* tests abnormal; CMI lowered—positive response only to 100 μg DNCB and one of the *in vitro* tests abnormal; CMI preserved—positive response to 50 μg or less DNCB, *in vitro* tests normal. Also for all the patients the immunoglobulins were determined by the Mancini method and antibodies against common viruses, measles and rubella, by the haemagglutination inhibition method.

### *Virus antigen and immunodiffusion test*

Human papilloma virus antigen was prepared from surgically removed skin warts as previously described (Pyrhönen & Penttinen, 1972). The antigen was prepared from a pool of wart tissue from more than ten patients and evidently contained various subtypes of papilloma virus (Pyrhönen, 1978a). Immunodiffusion (ID) tests were carried out by a micromodification of gel double diffusion (Pyrhönen & Penttinen, 1972).

### *ELISA (enzyme linked immunosorbent assay) for IgG and IgM antibodies against human papilloma virus (HPV)*

*Reagents.* (1) Guinea-pig gammaglobulin against human papilloma virus. Immune serum was prepared against purified human papilloma viruses extracted from a pool of human papilloma homogenates. The viruses were pelleted first in high speed centrifugation and further purified in a caesium chloride gradient (Pyrhönen & Penttinen, 1972). A guinea-pig was immunized by inoculating intramuscularly about $10^9$ purified HPV particles in 0·2 ml phosphate buffered saline (PBS), mixed with 0·2 ml complete Freund's adjuvant. After two boosters the immune serum was collected. Serum was first fractionated by $(NH_4)_2SO_4$ precipitation and further by DEAE-cellulose ion-exchange chromatography. For the assay the gamma fraction was diluted with PBS to a concentration of 50 μg protein/ml.

(2) HPV-antigen was prepared from a pool of human papilloma homogenates. Antigen was diluted with 0·05% Tween 20 in PBS (PBS-T).

(3) Positive and negative control sera and the sera analysed were diluted with PBS-T.

(4) Enzyme conjugates, swine antibodies against human IgG and IgM conjugated with alkaline

TABLE 1. Patients with epidermodysplasia verruciformis

| Patients No./initials/sex | Age (years) | Age of onset of disease (years) | HPV type | Malignant conversion | HPV antibodies by ID test | HPV antibodies by ELISA test | | RF activity | Non specific CMI* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | IgG | IgM | | | |
| | | *Familial cases* | | | | | | | | |
| 1/J.K./F | 38 | 5 | 5 | + | 0 | − | − | − | − | Sister of H.D. and T.G. |
| 2/H.D./F | 46 | 11 | 3 | − | 0 | − | − | − | − | Mother of E.D. and D.D. |
| 3/E.D./F | 22 | 7 | 3 | − | 0 | − | − | − | − | |
| 4/D.D./F | 21 | 7 | 3+5 | + | 0 | + | + | − | − | |
| 5/T.G./M | 52 | 5 | 5 | + | 1 | − | + | + | − | |
| 6/A.P./F | 25 | 10 | 3 | − | 16 | + | + | − | + | Her father had had flat warts on the hands since early childhood |
| 7/E.I./F | 18 | 4 | 3 | − | 0 | − | + | − | − | Father had had flat warts on the hands since early childhood |
| | | *Non familial cases* | | | | | | | | |
| 8/J.D./M | 43 | Early childhood | 3+5 | + | 0 | − | + | − | − | |
| 9/S.M./M | 22 | 8 | 5 | + | 8 | + | + | + | − | |
| 10/R.M./M | 36 | 2 | 5 | + | 0 | − | − | − | ± | |
| 11/J.G./M | 24 | 7 | 3 | − | 1 | + | − | − | − | |
| 12/M.G./M | 21 | 7 | 3 | − | 0 | − | + | − | ± | |

* (−) depressed; (±) lowered; (+) preserved.

phosphatase, were from Orion Diagnostica. Conjugates were diluted with 1% bovine serum albumin, 0·02% Tween 20, PBS solution. Optimal dilutions were found by titration.

*Assay procedure.* In the assay polystyrene cuvettes (Finnpipette Labsystems, Finland) were first coated with guinea-pig gammaglobulin against HPV. This was done by incubating 0·2 ml gamma solution (10 μg protein) in cuvettes at 37°C for 2 h. The cuvettes were then washed twice with PBS-T, first by rinsing only and then by allowing the PBS-T to stand for 10 min in cuvettes. Finally the cuvettes were washed twice with distilled water. Then 0·2 ml HPV-antigen solution was incubated in the cuvettes for 3 h at 37°C, which were then washed as set out above. To each cuvette 0·2 ml diluted sera was added and incubation continued for 2 h at 37°C. The dilutions used in the assay were 1:100, 1:500 and 1:2500. The cuvettes were then washed as described above and 0·15 ml enzyme conjugated dilutions were added to each and incubation continued overnight at room temperature in cuvettes. Washing was done again as above and finally alkaline phosphatase activity was measured. An aliquot of 0·15 ml paranitrophenylphosphate (Merck Art 6850) dissolved in diethanolamine buffer pH 10 (1 mg/ml) was pipetted into each cuvette. After 30 min incubation at 37°C, 0·25 ml 0·2 M NaOH was added to stop the reaction. Absorbances were measured (9 channel photometer, Finnpipette Analyzer System, Finnpipette Labsystems, Finland) at a wavelength of 405 nm.

In each test positive and negative serum controls were used. When a positive reaction was measured in the previous HPV assay the reactivity was further checked by absorbing the antibodies of the sera with original HPV antigen containing about $2 \times 10^{10}$ HPV particles/ml. Forty microlitres of serum, 60 μl PBS and 200 μl of virus antigen was incubated for 2 h in a tube at 25°C. Thereafter 3·7 ml PBS-0·05% Tween solution was added. Solutions were then centrifuged in SW 50·1 rotor, 35,000 rev/min for 1 h and finally reactivity was measured from this absorbed serum as well as from the original serum sample. Double measurements were made on each sample. A 20% or greater reduction in absorbance values was considered an indication of the presence of true antibody in the serum.

### *Latex test*

The latex test for rheumatoid factor(s) was performed using Hyland's RA test with latex globulin reagent. For the titrations U-microtitre equipment was used. Phosphate buffered saline was used as diluent. The plates were incubated at room temperature for 2–3 h before the results were read. Sera giving a positive reaction at a dilution of 1/8 were considered positive.

## RESULTS

The antibody findings, reactions in CMI tests, type of the HPV found in lesions as well as some clinical parameters of the EV patients are given in Table 1. Antibodies against HPV were detected in four of twelve patients (33%) with EV, while thirty of sixty-six (45%) of the control subjects with various types of warts had HPV antibodies measured by the ID method (Table 2). Some slight differences were also found in antibody prevalences within the control group. Using the ELISA method IgG class HPV antibodies were detected in four of twelve (33%) patients with EV (Table 2) and IgM class HPV antibodies in seven of the patients (58%). In the control group IgG and IgM HPV antibodies were detected in 44% and 71%, respectively. The frequency and titres of measles and rubella antibodies were, in all groups, similar to those in age-matched healthy controls of the Polish population. In all patients' sera immunoglobulins were normal.

Non-specific cell-mediated immunity was markedly depressed in a majority of patients with EV while in patients with plane and common warts as well as in patients with plantar or genital warts the non-specific CMI was most preserved (Table 3).

TABLE 2. Antibodies in patients with EV and the control group with other HPV diseases

| Group | No. of patients | HPV antibodies by ID test | | HPV antibodies by ELISA test IgG | | IgM | | Patients with RF activity | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % |
| EV HPV 3 | 6 | 2 | 33 | 2 | 33 | 3 | 50 | — | — |
| EV HPV 5 | 4 | 2 | 50 | 1 | 25 | 2 | 50 | 2 | 50 |
| EV HPV 3+5 | 2 | — | — | 1 | 50 | 2 | 100 | — | — |
| EV (total) | 12 | 4 | 33 | 4 | 33 | 7 | 58 | 2 | 17 |
| Plane warts | 9 | 4 | 44 | 4 | 44 | 8 | 89 | 2 | 22 |
| Common warts | 42 | 17 | 40 | 17 | 40 | 28 | 67 | 12 | 28 |
| Plantar warts | 4 | 3 | 75 | 1 | 25 | 4 | 100 | — | — |
| Condymata acuminata | 11 | 6 | 55 | 7 | 64 | 7 | 64 | — | — |
| Control group (total) | 66 | 30 | 45 | 29 | 44 | 47 | 71 | 14 | 21 |

TABLE 3. Non-specific cell-mediated immunity in patients with EV and the control patients

| Group | No. of patients | No. of patients with CMI Preserved | Lowered | Depressed |
|---|---|---|---|---|
| EV | 12 | 1 | 2 | 9 |
| Plane warts | 15 | 10 | 3 | 2 |
| Common warts | 15 | 13 | 2 | — |
| Plantar warts | 15 | 11 | 3 | 1 |
| Condylomata acuminata | 14 | 13 | — | 1 |

## DISCUSSION

In the present study antibodies against pooled HPV were measured by two principal methods: immunodiffusion and enzyme linked immunosorbent assay. Both the methods gave somewhat lower HPV antibody prevalences in patients with EV than in the control group with other types of wart diseases. By the ELISA test antibody classes could also be determined; about half the EV patients had antibodies of IgM type and less frequently of IgG type. Tests for IgM antibodies may in some cases give false positive reactions due to IgM rheumatoid factors. Rheumatoid factors checked by the latex test were, however, found with the same frequency in the EV patients' sera as in the control patients' sera.

The HPV genome has been shown to be heterogeneous. Various genome structures (Gissmann, Pfister & zur Hausen, 1977) and distinct polypeptide compositions for HPV proteins have been found (Gissmann *et al.*, 1977; Orth, Favre & Croissant, 1977; Pfister, Gissman & zur Hausen, 1977; Staquet *et al.*, 1978). Also different serological HPV types have been described (Almeida, Oriel & Stannard, 1969; Gissmann *et al.*, 1977; Orth *et al.*, 1978a, b). According to the newly accepted classification at least seven different HPV subtypes have been isolated—HPV-1a, HPV-1b, HPV-1c, HPV-2, HPV-3, HPV-4 and HPV-5 (Coggin & zur Hausen, 1979). The viruses isolated from EV lesions have been classified into either subtype 3 or 5. Due to this heterogenity one could insist that the viruses inducing EV might be antigenically different from the papilloma viruses used in the present study (Pass *et al.*, 1977) and consequently the resulting antibodies were not measurable. Indeed, it was shown that among the twelve EV patients studied lesions of six patients were induced by HPV 3, four by HPV 5 and two were infected by both HPV 3 and 5 (Orth *et al.*, 1978c). This distribution does not explain the slight serological hyporeactivity detected against pooled HPV: in other studies 50–80% of adult subjects independent of whether they have or do not have overt wart disease (Pyrhönen, 1978a), as well as in the present control patients with other types of warts, have been shown to have antibodies measurable by ID. This is a higher proportion than that found among patients with EV. Furthermore, both the patients with EV induced by type 3 and 5 viruses had IgM type antibodies suggesting that the pooled antigen evidently contained both types of HPV antigenicity. Finally, the possibility of general impairment in antibody reactivity among EV patients was largely excluded by detecting normal immunoglobulin levels and normal antibody levels against rubella and measles virus in their sera.

The importance of the various immunological factors in the appearance and regression of warts is still not completely clear. The appearance of complement fixing IgG type antibodies against the virus in a proportion of patients during the regression of warts (Pyrhönen & Johansson, 1975) suggests that especially in the early phase of the disease viral antibodies might assist in wart rejection. Although four of the twelve EV patients had IgG class antibodies measured by ELISA, none of them had such antibody levels that would fix complement in the complement fixation test used (Pyrhönen & Penttinen, 1972). Cell-mediated reactions measured by *in vitro* tests like lymphocyte transformation and migration inhibition tests have also been detected against human wart-virus (Morison, 1975) and perhaps also against some wart-associated cellular antigens (Lee & Eisinger, 1976). Cellular mechanisms may thus be involved in rejection of HPV tumours. Since in the present study tests of non-specific cell-mediated immunity showed the most remarkable difference between the patients with EV and other types of warts (Glinski *et al.*, 1976), it is evident that impairment in cell-mediated reactivity is one of the most important factors contributing to a chronic HPV infection like EV with potentialities to malignant conversion.

The conclusions from the present study are that sometimes antibody reactions against HPV might be deficient in EV patients, but that this is not a general rule. More remarkable is a defect in cell-mediated immunity, perhaps also specifically against HPV or some yet unknown HPV associated cellular antigen(s). Malignant conversion, according to a study by Orth *et al.* (1978b) and also included in the present study, seems to be dependent mainly on the type of the inducing virus; malignant change is especially associated with HPV 5. Further studies on the immunological factors in EV disease are needed, however, before a policy for prevention and treatment of EV can be defined.

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# The quantification and significance of mast cells in lesions of Behçet's disease

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## SUMMARY

Mast cells in the upper dermis and mucosal subepithelial layer were counted in thirty consecutive light microscopy oil-immersion fields of the following: thirty-four skin and oral specimens from lesions in patients with active Behçet's disease, eight specimens of apparently uninvolved skin of the same patients, and 102 lesions of a variety of other skin diseases. The results revealed a significant increase in the number of mast cells in Behçet's lesions. Furthermore, the histamine content of five reactive lesions showed a two-fold increase compared with that in apparently uninvolved skin of patients with active Behçet's disease.

It is suggested that mechanisms similar to cutaneous basophil hypersensitivity may be involved in the production of Behçet's lesions.

The phenomenon of skin hyperreactivity in Behçet's disease (BD) is unique and unexplained. Twelve to 48 h following intracutaneous injection of normal saline or a needle prick, an erythematous induration develops (Blobner, 1937; Jenson, 1941; Katzenellenbogen, 1968; Sobel *et al.*, 1973).

Lehner (1969) described a three-fold rise in the number of mast cells in the cellular infiltrate of recurrent oral ulcers, including BD. We, likewise, showed a significant increase of mast cells in the infiltrate of naturally occurring mucocutaneous lesions of BD as well as in the reactive inflammatory response (Haim *et al.*, 1976). The presence of mast cells in large numbers in new reactive lesions suggested a possible role in the production of the cutaneous response.

In an attempt to evaluate the significance and possibly the specificity of mast cells in BD, we conducted a comparative study of their number in provoked as well as in naturally occurring mucocutaneous lesions, in apparently normal skin of patients in the active stage of the disease, and in a variety of other inflammatory and proliferative skin diseases.

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## MATERIAL AND METHODS

The number of mast cells in forty-two biopsy specimens from patients in the active stage of BD was counted. The specimens included: twenty-one reactive lesions induced on the forearms by needle-prick and biopsied arbitrarily after 24 h in the presence of an erythematous induration; eight from apparently unaffected forearm skin; five from spontaneous, newly developed oral ulcers (three minor and two major); and eight from spontaneous cutaneous lesions (two erythema nodosum-like, four scrotal ulcers and two papulo-follicular lesions). The number of mast cells in 102 specimens from lesions of a variety of other clinically and histologically proven skin disorders was similarly determined and the results were compared and statistically evaluated utilizing the Student's '*t*'-test. Details of the skin disorders studied are given in Table 1.

TABLE 1. Mast cells in a variety of skin diseases

| Disease | No. of cases | No. of cells | Significance* |
|---|---|---|---|
| Granuloma annulare | 12 | 47 | $P < 0.05$ |
| Chronic dermatitis | 14 | 42 | $P < 0.05$ |
| Dermatitis herpetiformis | 4 | 40 | $P < 0.05$ |
| Pemphigus | 4 | 37 | $P < 0.05$ |
| Sarcoidosis | 9 | 36 | $P < 0.01$ |
| Bullous pemphigoid | 4 | 35 | $P < 0.01$ |
| Psoriasis | 8 | 29 | $P < 0.01$ |
| Lichen planus | 3 | 26 | $P < 0.01$ |
| Scleroderma | 6 | 26 | $P < 0.01$ |
| Leishmaniasis | 8 | 29 | $P < 0.01$ |
| Tuberculosis | 6 | 23 | $P < 0.01$ |
| Pityriasis lichenoides | 6 | 22 | $P < 0.01$ |
| S.L.E. | 6 | 20 | $P < 0.01$ |
| Acute vasculitis | 5 | 19 | $P < 0.01$ |
| Erythema nodosum | 5 | 19 | $P < 0.01$ |

* Between disease and pathergic lesions.

The diagnosis of BD was based upon the presence of at least two of the three cardinal and one of the other manifestations recognized as part of the process. In all cases the biopsy was performed under local procaine hydrochloride analgesia, taking care to include a surrounding rim of normal skin. The tissue was fixed in 10% formalin, embedded in paraffin and from each specimen sections were stained with haemoxylin-eosin, toluidine blue and with naphthol-AS-chloroacetate NCA (Leder, 1964). The latter stain gave better identification of mast cells.

In all specimens the number of mast cells was counted in thirty consecutive immersion fields of the upper dermis and of the mucosal subepithelial layer. Only intact mast cells were counted.

In addition, the histamine in five reactive lesions was estimated by the enzymic-isotopic method (Taylor & Snyder, 1972) and the results were compared with those found in five specimens from apparently normal contralateral skin obtained at the same time from the same patients.

## RESULTS

The mean numbers of mast cells counted in lesions of BD were as follows: 101 in spontaneous oral lesions, 57·4 in spontaneous cutaneous lesions and 62 in reactive skin lesions. These values are significantly higher than the mean number of mast cells (25·5) counted in uninvolved skin of patients with active Behçet's disease ($P<0{\cdot}01$, $<0{\cdot}05$ and $<0{\cdot}01$ respectively) (Table 2). The mean numbers of mast cells found in lesions of other skin diseases are shown in Table 1. Apart from urticaria pigmentosa the number was significantly lower than in reactive Behçet's lesions (the $P$ values range from $<0{\cdot}01$ to $<0{\cdot}05$) and was in most of these disorders similar to that for uninvolved skin of active Behçet's disease.

The mean content of histamine in five specimens of Behçet's reactive lesions was 43 ng/mg tissue. This is two-fold higher than the mean content of 21 ng found in five control skin specimens taken from uninvolved contralateral areas of the same patients (Table 3).

TABLE 2. Mast cell quantification in Behçet's disease

| Specimen | No. of cases | No. of cells | Significance* |
|---|---|---|---|
| Normal skin | 8 | 25·5 | |
| Oral lesions | 5 | 101·0 | $P<0{\cdot}01$ |
| Skin lesions | 8 | 57·4 | $P<0{\cdot}05$ |
| Pathergy | 21 | 62·0 | $P<0{\cdot}01$ |

* Between Behçet's lesions and normal skin of active Behçet's patients.

TABLE 3. Histamine quantification in Behçet's disease (enzymic-isotopic method)

| Patient | Normal skin (ng/mg tissue) | Pathergic lesions (ng/mg tissue) |
|---|---|---|
| M.S. | 20 | 21 |
| S.S. | 20 | 40 |
| C.D. | 20 | 48 |
| M.C. | 17 | 30 |
| G.H. | 28 | 78 |
| Mean | 21 | 43 |

## DISCUSSION

The data in this study show a significant increase in the number of mast cells in the reactive and in the spontaneous lesions of BD as compared with uninvolved skin of patients in the active stage of the disease and with other skin diseases. Our data also revealed a two-fold increase in the concentration of histamine in the lesions.

In accordance with the literature (Nazzaro, 1966), in our cases the predominant cells in the exudate were lymphocytes and other mononuclear cells. Neutrophils were occasionally present (Haim *et al.*, 1976) and eosinophils absent or rare. Mast cells, on the other hand, were comparatively abundant and their role in this process is yet to be decided.

The cutaneous basophil hypersensitivity reaction (Dvorak & Dvorak, 1972; Askenase, 1973, 1977; Dvorak, 1976; Askenase, Haynes & Hayden, 1976) is a form of cell mediated immunity which differs from the classical tuberculin type by being induced by several antigens in the absence of Freund's adjuvant. The basophils in this process are attracted to the inflammatory area by various specific chemotactic factors (Ward *et al.*, 1975; Askenase *et al.*, 1976; Dvorak, 1976; Askenase, 1977; Hastie, Levy & Weiss, 1977). The question arises whether a similar mechanism exists in BD in which mast cells replace the basophils.

Askenase (1977) considered both mast cell and basophil to have similar and complementary functions. Dvorak, Mihm & Dvorak (1976) described an increase in the number of mast cells in allergic dermatitis in man and considered it an example of cutaneous basophil hypersensitivity. This reaction has been demonstrated in vaccinia virus infection (Dvorak & Hirsch, 1971) and it may be relevant that among the aetiological hypotheses suggested in BD, the viral one is still considered (Sezer, 1953; O'Duffy, Corney & Deodhar, 1971). Moreover, the complement system plays an important role in chemotaxis (Ward *et al.*, 1975) and there is evidence that in most cases of BD there is increased total haemolytic complement activation (Kogure, Shimada & Hara, 1971) as well as a striking increase in serum levels of the ninth component of complement (Kawachi-Takahashi *et al.*, 1974).

We feel justified in speculating that the role which we are ascribing to mast cells in BD may offer an explanation for the protean manifestations met with in this process.

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# Liver changes in porphyria cutanea tarda patients treated with chloroquine

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## SUMMARY

In thirty-four patients with porphyria cutanea tarda treated with small doses of chloroquine, liver biopsies were performed before and after treatment. In seventeen cases (50%) the morphological patterns before treatment corresponded to unstabilized fibrosis, while in eleven (32·4%) there were non-specific changes in the form of focal fatty change, haemosiderosis, and a mild fibrosis of the portal tracts. Active chronic hepatitis was found in three patients (8·8%), and cirrhosis also in three cases.

Although in all patients a clinical and metabolic remission of porphyria cutanea tarda occurred during treatment, the morphological patterns in the liver parenchyma remained on the whole unchanged. Only in five cases was there an increase in the inflammatory changes, while in two patients these changes tended to disappear.

It has been generally accepted that both phlebotomy therapy and, more recently, chloroquine administration (Taljaard *et al.*, 1972; Malina & Chlumský 1973; Gebel & Doss, 1978) lead to clinical and metabolic remission in the vast majority of porphyria cutanea tarda (PCT) cases. While it has been confirmed that repeated phlebotomies do not damage the liver parenchyma in PCT (Chlumský, Chlumská & Malina, 1973; Malina, Chlumský & Chlumská, 1974), the effect of chloroquine on the function and morphology of hepatic tissue is not yet sufficiently known. Several opinions have been expressed on the possible impairment of the liver parenchyma due to the drug (Cripps & Curtis, 1962; Sweeney *et al.*, 1965; Bloom, 1969; Reed, 1974).

Our paper deals with functional and structural liver changes observed in PCT patients treated with chloroquine.

## MATERIAL AND METHODS

We studied thirty-four patients—twenty-seven men aged between 29 and 65 (mean 46·9) years and seven women aged 28–65 (mean 60·2) years. All were treated with chloroquine (Delagil, G.Richter,

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Budapest), administered in small doses of 125 mg twice weekly for 3–8 months. Eight of these patients had earlier been successfully treated with repeated phlebotomies; they were given chloroquine following a relapse of the disease. Before and after the chloroquine treatment all patients underwent thorough clinical and laboratory examinations. A punch biopsy of the liver was performed according to Menghini's technique. The first specimen of hepatic tissue was taken soon after the appearance of the first clinical signs in eighteen patients, while in sixteen patients, either untreated or having previously been the subject of phlebotomy therapy, within a period of 1–12 years. Chloroquine therapy was then started within 2 weeks after liver biopsy. Repeat liver biopsy was performed after the termination of chloroquine treatment, in twenty-three patients within the first 2 months, in another ten patients within the first year, and in one patient after 2 years delay.

The specimens were fixed in 10% neutral formol and processed by the usual paraffin technique. For staining we used van Gieson's and H & E method, aldehyde fuchsin and Perl's method for iron. Unfixed specimens were examined in Wood's light for the presence of porphyrins.

## RESULTS

A history of viral hepatitis was found in seven of our patients, syphilis treated with heavy metals in three, tuberculosis treated by antituberculous drugs in four, diabetes mellitus in four and excessive alcohol intake (mostly of beer, less frequently of wine and spirits) in twenty-two cases. These factors have not only a hepato-toxic but also a porphyria-inducing effect. It was possible to find them separately or in various combinations especially in the patients with liver fibrosis, chronic hepatitis and hepatic cirrhosis. It is therefore not surprising that the serum transaminases were often somewhat raised even before the commencement of the therapy.

At the beginning of chloroquine treatment an increase of serum transaminases was observed in almost all PCT patients, but not above four to five times the normal values. As a result of treatment they never exceeded 150 u/l (Figs 1 and 2). In no case did we observe bilirubinaemia above 2 mg% or arthralgia, cephalalgia, dyspepsia or any other signs of chloroquine intolerance. Nor did we observe hepatomegaly or splenomegaly as a result of therapy. Mild hepatomegaly was nevertheless present in fourteen patients before the beginning of chloroquine treatment, in the majority of biopsy-confirmed cases of liver fibrosis, and almost always in the cases of chronic hepatitis. Mild splenomegaly was

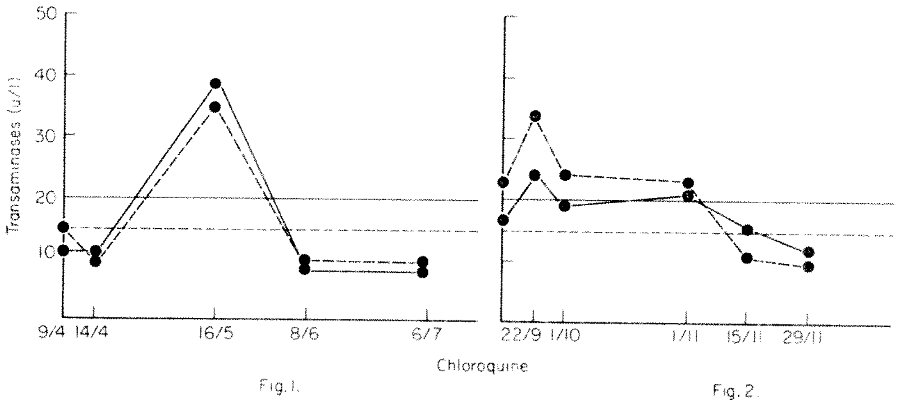


FIGURE 1. Typical changes in transaminases in a patient with non-specific changes on liver biopsy, during the course of chloroquine treatment.

FIGURE 2. Similar changes in transaminases in a patient with unstabilized liver fibrosis. (—) SGPT; (- - -) SGOT.

present in only two subjects. It can be generally said that the liver function tests corresponded to the grade of histopathological changes found. The serum iron levels did not correspond to the extent or the degree of liver siderosis or to the progression of liver changes.

Table 1 shows the laboratory values before and after treatment. It can be seen that the results of these tests did not significantly change even in those patients with more advanced types of liver lesion.

TABLE 1. Laboratory findings in PCT patients before and after treatment with chloroquine

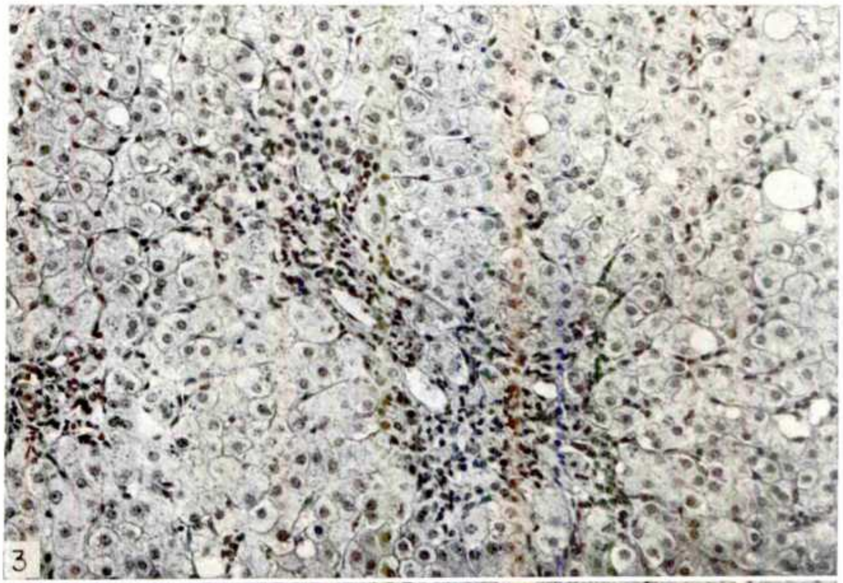
| | No. of patients | No. of patients with abnormal values | |
|---|---|---|---|
| | | Before treatment | After treatment |
| SGOT | 34 | 22 | 11 |
| SGPT | 34 | 24 | 13 |
| Serum bilirubin | 34 | 2 | 2 |
| Serum gamma-globulin | 34 | 12 | 10 |
| BSP-clearance | 33 | 19 | 15 |
| Serum iron | 33 | 28 | 17 |

TABLE 2. Histopathological findings in PCT patients before and after treatment with chloroquine

| Before treatment | | | | After treatment | | | |
|---|---|---|---|---|---|---|---|
| No. of patients | Histopathological findings | Steatosis | Siderosis | No. of patients | Histopathological findings | Steatosis | Siderosis |
| 11 | Non-specific | 10 | 7 | 9 | Non-specific | 7 | 6 |
| 17 | Unstabilized fibrosis | 14 | 12 | 18 | Unstabilized fibrosis | 15 | 8 |
| 3 | Chronic hepatitis | 2 | 2 | 4 | Chronic hepatitis | 3 | 1 |
| 3 | Cirrhosis | 2 | 2 | 3 | Cirrhosis | 2 | 2 |
| 34 | | 28 | 23 | 34 | | 27 | 17 |

Biopsies (Table 2) before treatment revealed non-specific changes in the liver parenchyma in the form of slight fibrosis of the portal tracts and focal hyperplasia of Kupffer cells in eleven patients (32·4%). In some areas hepatocytes showed irregularities in the size of nuclei, which were occasionally vacuolated in the periportal regions, and a focal, predominantly large-drop fatty change with on occasion haemosiderosis of Kupffer cells and hepatocytes.

In seventeen patients (50%) the changes in pre-treatment biopsies were more marked. Portal tracts were enlarged and fibrotic with infrequent pseudocanaliculi around their peripheries and a slight diffuse round-cell inflammatory infiltration (Fig. 3). Fine fibrous streaks could be seen in the form of so-called septal fibrosis, penetrating from the slightly angulated periportal areas into the surrounding parenchyma. There was a not very marked diffuse or focal hyperplasia of Kupffer cells. Hepatocytes showed, besides a slight nuclear irregularity, focal large-drop fatty change and sometimes haemosiderosis. Only rarely did we observe their unicellular necrosis (Fig. 4). We evaluated this finding as mild unstabilized fibrosis.



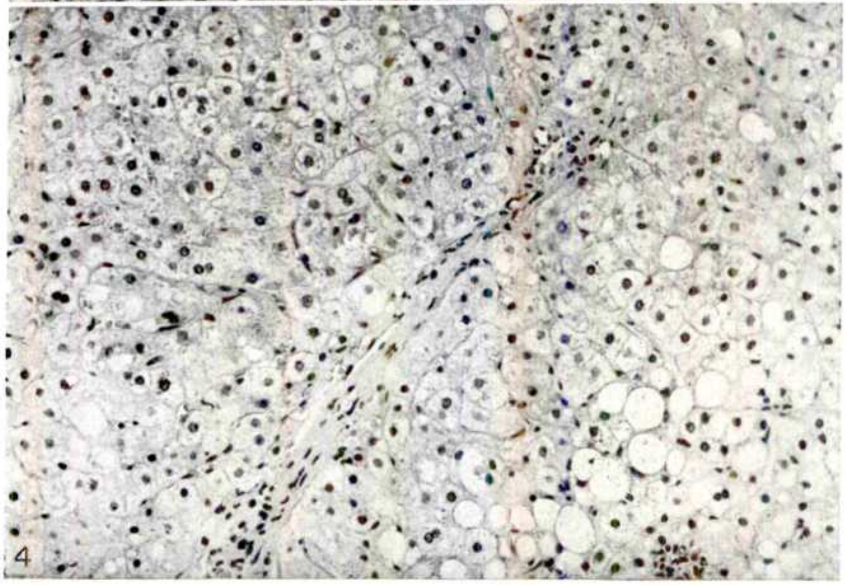


FIGURE 3. Unstabilized fibrosis: mild fibrosis and round-cell inflammatory infiltration of enlarged portobiliary space with slight hyperplasia of Kupffer cells (H & E, × 140).

FIGURE 4. Septal fibrosis and focal large-droplet steatosis with resorptive nodule in the periphery of the picture. Slight hyperplasia of Kupffer cells (H & E, × 140).

Active chronic hepatitis was diagnosed in three patients (8·8%). A pseudoacinous type of cirrhosis with signs of activity was found also in three patients.

The porphyrin test in Wood's light was performed on only some of the liver biopsies (fifteen cases) and was always positive. This finding disappeared after the treatment in all liver samples investigated.

Transition of non-specific changes into a mild unstabilized fibrosis was observed in four patients in the biopsies repeated after chloroquine treatment, while in another patient with a mild unstabilized fibrosis we found changes corresponding to chronic active hepatitis. However, in two patients with an unstabilized fibrosis the inflammatory changes disappeared. In the remaining cases the morphological patterns did not change during the treatment.

Repeated biopsies revealed that haemosiderin disappeared in seven of the twenty-three cases of liver haemosiderosis found during the initial examination. In one instance, however, haemosiderin was found only in the specimen obtained after the treatment. The incidence of fatty change also varied slightly: it was absent three times in repeated biopsies, while in two specimens it appeared only after treatment.

## DISCUSSION

Histopathological liver changes found in PCT may differ in type (Braun & Berman, 1959; Creutzfeld *et al.*, 1966; Thaler, 1968; Wepler & Wildhirt, 1968) and are not directly related to the existing clinical signs of the disease. They show a dependence on the patient's age and possibly on other unfavourable influences (Chlumský *et al.*, 1973; Malina *et al.*, 1974). Repeated phlebotomies, which remove excessive stores of prophyrins and iron, cannot be performed under certain circumstances. That is why the use of this treatment in PCT was to a certain extent reduced, and some authors recommended chloroquine to achieve the same therapeutic effect. It was ascertained by that time that this drug releases porphyrins from their liver stores and stimulates their excretion in the urine. Soon, however, it became obvious that higher doses of chloroquine can give rise to fever, malaise, headache, nausea, constipation and abdominal pain, and for some time the attitude to this new therapeutic approach was rather cautious (Cripps & Curtis, 1962; Sweeney *et al.*, 1965; Bloom, 1969; Reed, 1974).

Yet the small doses of chloroquine administered to our patients—125 mg twice weekly for not more than 8 months—had no undesirable side-effects, except for the slight rise in the serum transaminases during the first 2 weeks of treatment. Laboratory tests after the treatment confirmed a significant decrease, usually even a return to normal values. The levels of gamma-globulins and bromsulphthalein clearance were less likely to recede (Malina, 1977; Chlumský *et al.*, 1977; Lehr & Saenz, 1978).

Morphological liver changes were most varied and, as already stated above, we found no relation with the duration of the PCT. Focal fatty change and mild haemosiderosis of hepatocytes, often accompanied by a mild fibrosis of portal tracts, were the mildest forms. Morphological and laboratory examinations showed little changes in this state although in four patients it changed during the period under observation into a mild unstabilized fibrosis. Therefore, not even this type of change allows a completely favourable prognosis. On the basis of our long experience with biopsies we call fibrotic changes with mild inflammatory signs 'mild unstabilized fibrosis'. It was the most frequent finding in our group of patients and repeated examinations spoke for its continued mild progression. In only two patients from this group did the inflammatory signs disappear during treatment, while a slight fibrosis of the portal tracts remained. Active chronic hepatitis was diagnosed three times; its viral aetiology was established only once, when HBsAg was found in the plasma, although altogether eight patients indicated in their case history that they had at some time or other had viral hepatitis. Cirrhotic transformation of the pseudoacinous type was identified three times.

The incidence of focal fatty change varied with repeated biopsies, as occurs in other pathological states. Thus, it is impossible to assess unequivocally its extent in relation to either the progression or recession of histopathological liver changes. In no case were fatty changes of a diffuse type observed.

Haemosiderosis, never more then mild, was present before the chloroquine treatment in twenty-three cases. Repeated biopsies confirmed it in only seventeen samples. It usually receded as sideraemia decreased.

Regarding the role of liver haemosiderosis in the aetiology of PCT we suggest, in agreement with the results of other authors (Lehr & Saenz, 1978), that it is only secondary. Hypersideraemia and hepatic siderosis can be found in only some PCT patients, and there is no direct relationship between plasma iron levels and hepatic iron stores. It follows from these facts and from the favourable therapeutic effect of chloroquine in the vast majority of PCT cases that its mechanism can only partially depend on the effect of the drug on hepatic iron metabolism.

With the exception of haemosiderosis, morphological liver changes remained, on the whole, unchanged during treatment. In cases showing improvement or worsening of findings, the possibility of an unrelated change in the hepatic lesion cannot be excluded: exact assessment is impossible, as the dynamics of the hepatic changes in untreated patients are not known. It was observed, however, that before any symptomatic therapy for PCT existed, the incidence of chronic hepatitis and/or hepatic cirrhosis was more frequent among PCT patients. If, as was originally thought, pathological changes in liver tissue of PCT patients are the result of an increased formation and accumulation of porphyrins in hepatocytes (Braun & Berman, 1959; Creutzfeldt *et al.*, 1966; Wepler & Wildhirt, 1968), then treatment with chloroquine, which temporarily releases them (Scholnick, Epstein & Marver, 1973), should favourably influence even the microscopic changes. Our findings, however, show that although this treatment is suitable for achieving a reliable clinical and metabolic remission, it does not involve fundamental histopathological changes once the prophyrins in hepatic tissue have been reduced. Moreover chloroquine, administered in low doses, does not result in a significant impairment of liver parenchyma.

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# The demonstration of bacteria on and within the stratum corneum using scanning electron microscopy

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## SUMMARY

Using scanning electron microscopy it has been possible to demonstrate the location of bacteria on and within the stratum corneum of the human foot. Biopsies taken either by sectioning or by removing stratum corneum with cyanoacrylate ester adhesive were examined using a Jeol JSM-T20 scanning electron microscope. Bacteria could be seen easily on specimens from skin which had been occluded to increase the number of bacteria present. On the surface, bacteria were scattered widely in small colonies (usually containing less than ten bacteria). Although bacteria could be seen around the orifice of sweat ducts they did not preferentially favour these sites. Within the stratum corneum, bacteria could be found as relatively large colonies but these were usually associated with sweat ducts or the underside of the furrows in the skin surface. This study suggests that, in normal skin, bacteria are able to colonize both the surface and the depths of the stratum corneum.

Despite an abundance of information in the literature concerning the number and type of micro-organisms to be found on the skin, there is a relative dearth of reliable data concerning their location.

It is generally believed that bacteria are confined to the superficial layer of the stratum corneum (Kligman, 1965) although Updegraff (1964) was able to show bacteria present even after fourteen tape strippings at the same site. Noble (1968), however, claimed that the majority of bacteria could be removed by four strippings. These differences may of course be readily explained by the fact that the authors were examining different sites, and in addition tape stripping gives only a poor estimate of the depth of penetration since it will often fail to sample furrows in the skin (Kligman, 1965).

Although Montes & Wilborn (1969, 1970) using transmission electron microscopy have shown bacteria within the stratum corneum they have claimed that the infundibula of hair follicles are the sites at which most bacteria are located. Puhvel, Reisner & Amirian (1975), however, found relatively low numbers of aerobic staphylococci in pilosebaceous follicles and suggested that these bacteria reside principally on the surface of the skin. Similarly Kligman (1965) showed that the sebaceous duct and associated infundibulum of normal scalp skin was usually free from bacteria although the sebaceous, i.e. hairless, follicles of the face were inhabited by large numbers of bacteria.

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There are conflicting reports about the role of the eccrine sweat duct as a location of bacteria. Lovell (1945) and Montes & Wilborn (1970) failed to find bacteria in sweat ducts whilst O'Brien (1950), Williams (1963) and Hölzle & Kligman (1978) have provided evidence which suggests that sweat ducts may normally contain bacteria.

It is difficult to correlate the work which has been done on the location of skin bacteria, since so many different sites have been examined. It is likely that the structure of the stratum corneum and the presence or absence of hair and exocrine glands will have a significant influence. A further important factor will be the favourability or otherwise of conditions for bacterial multiplication.

In this present study a single site has been examined under conditions which favour bacterial multiplication and under conditions which do not.

## MATERIALS AND METHODS

### *Occlusion*

The foot was chosen as a suitable site for investigation because the calloused region enabled us to take biopsies without causing distress to the subject. In order to produce conditions for the visualization of bacteria the foot was occluded with Cling Film (Safe Wrap Cling Film, Marchant Manufacturing Company Limited) using a modification of the method of Marples & Kligman (1974). It is recognized, however, that this may produce abnormal conditions.

Subjects were instructed to wash their feet in the morning using a non-germicidal soap and then to put on a clean pair of socks. In the afternoon of the same day an area of approximately 5 cm × 5 cm situated below the ankle bone on the lateral surface of the foot and extending onto the sole was occluded with Cling Film. The Cling Film was sealed to the surface with waterproof Elastoplast (T.J. Smith and Nephew Limited) and protected from damage by covering with a layer of Blenderm Surgical Tape (3M United Kingdom Limited). The occlusive dressing was left on for 2 days and then removed so that the occluded site could be sampled.

### *Biopsy*

Biopsies were taken from the cornified area of the foot. The skin surface was lifted using a mounted needle, and a razor blade was used to slice off a small sliver of skin above the needle.

### *Skin surface biopsy (SSB)*

Skin surface biopsies were taken using a modification of the method devised by Marks & Dawber (1971). A small drop of cyanoacrylate ester adhesive (Super Glue 3 (TM), Woodhill Chemical Sales Corporation) was placed on an aluminium stub used for mounting electron microscopy specimens. This was placed firmly onto the surface of the skin and left in place for 60 s; then, using an even pull, the stub was removed together with a layer of adhering stratum corneum. SSBs were taken from cornified areas on the sole of the foot and from non-cornified areas on the lateral surface.

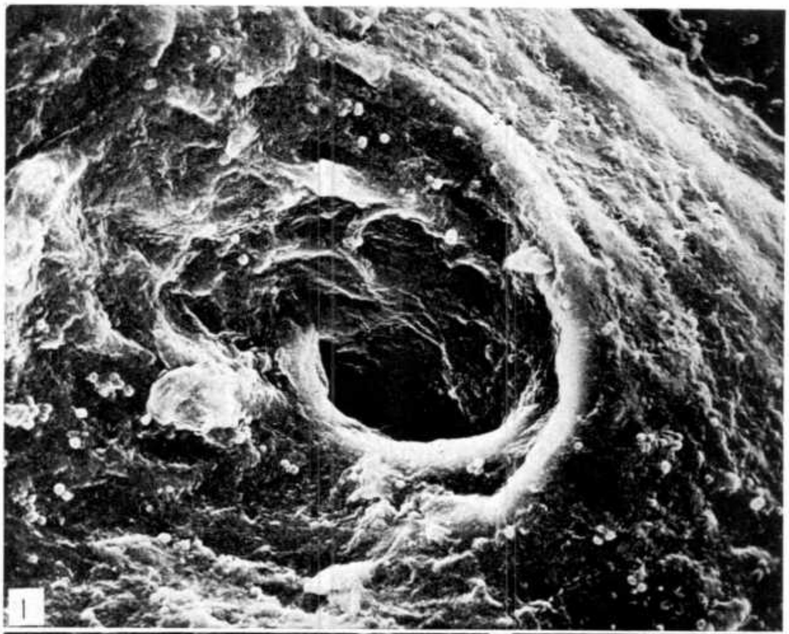
### *Electron microscopy*

Both biopsies and SSBs were sputter coated with gold using the Polaron scanning electron microscope coating unit E.500 and then examined in the JSM-T20 scanning electron microscope (Jeol Ltd).

## RESULTS

### *Biopsy*

*Occluded Skin.* The areas examined were supplied with large numbers of easily visible sweat ducts.



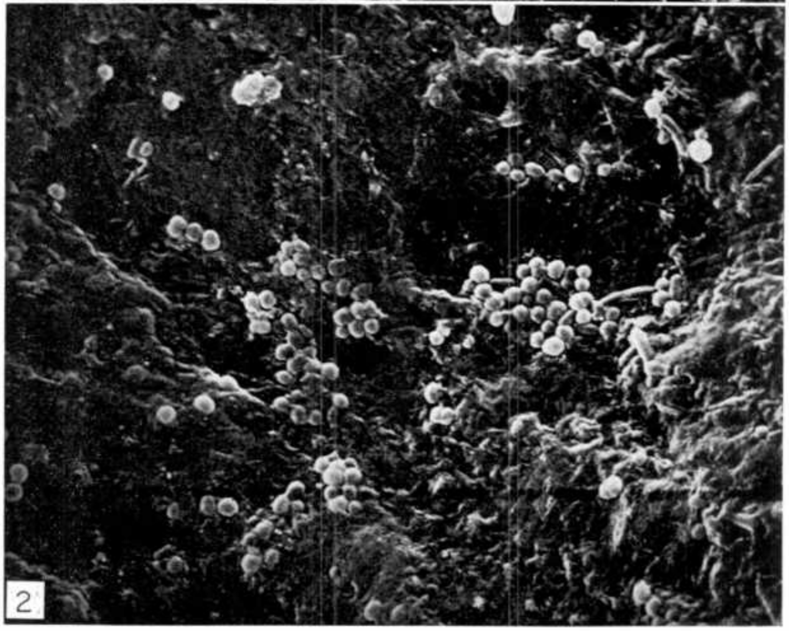
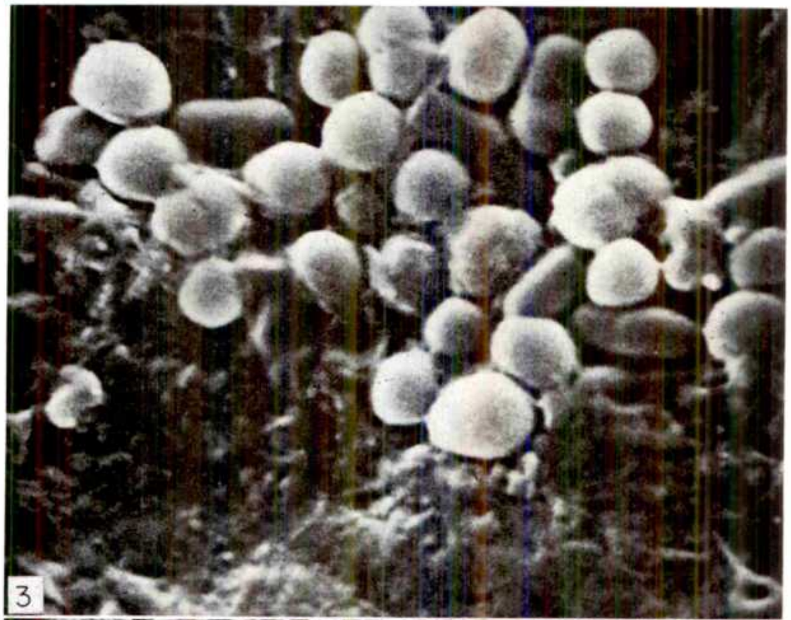


FIGURE 1. Biopsy: opening of a sweat duct. Note the scattered bacteria (× 960).
FIGURE 2. Biopsy: small bacterial colonies on the skin surface (× 2100).

Most sweat ducts had distinct openings (Fig. 1). The skin surface showed little detail of the structure of the corneocytes of which it was composed, appearing to be covered with an amorphous film. Bacteria were scattered widely over the surface (Fig. 1) either singly or in small colonies. The colonies rarely contained more than thirty to forty bacteria (Fig. 2) although occasionally some larger colonies



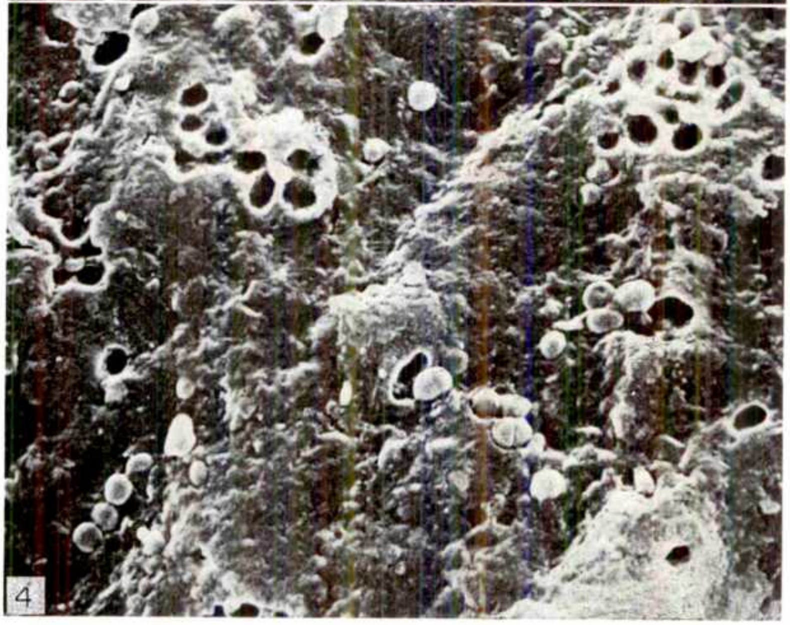
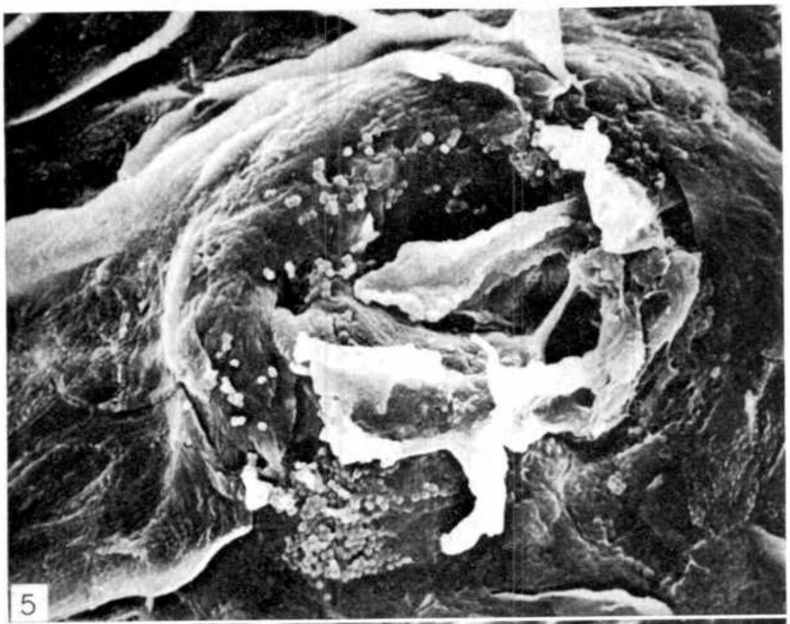


FIGURE 3. Biopsy: a small colony of cocci containing some rod-shaped bacteria (× 1200).

FIGURE 4. Biopsy: pitting in the surface of corneocytes apparently caused by bacteria (× 3700).

were seen. Most of the bacteria were coccoidal in shape although rod-shaped bacteria were sometimes seen (Fig. 3). (When swabs were taken from occluded foot only micrococcaceae were isolated.) The bacteria did not appear to favour any particular site, being distributed more or less randomly over the surface. Bacteria were seen surrounding sweat ducts and some small colonies were seen within the



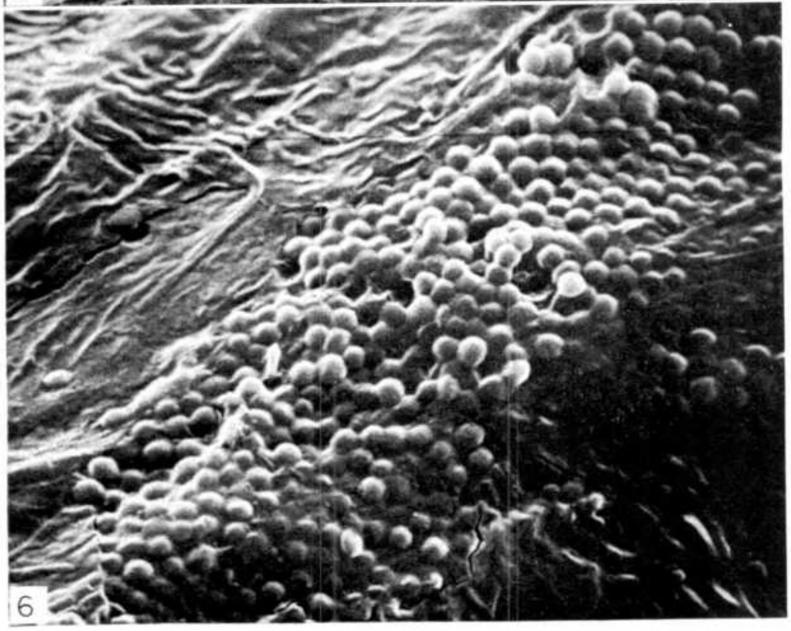


FIGURE 5. SSB: sweat duct opening projecting above surface. Note colonies within lumen and on surrounding cells, also pitting of cell surface (× 1200).

FIGURE 6. SSB: large colony on underside of skin furrow (× 4000).

openings of the ducts. In many areas bacteria appeared to have made pits in a superficial film (Fig. 4). Some bacteria could also be seen partially submerged in this film.

*Unoccluded skin.* Biopsies taken from the same site on an unoccluded foot were similar in appearance except that there were fewer bacteria to be seen. Those bacteria which were present were randomly

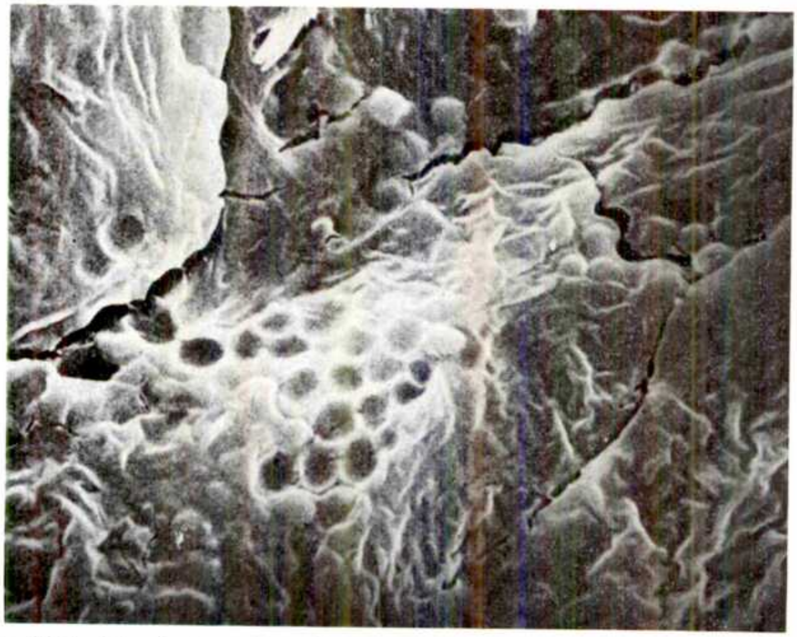


FIGURE 7. SSB: pitting in the surface of a corneocyte, apparently caused by bacteria (× 4900).

scattered about the surface and they could occasionally be seen associated with sweat ducts, although, as with the biopsy taken from the occluded foot, they were not concentrated at these sites.

*Skin surface biopsy*

*Occluded skin.* Unlike the biopsy there was no superficial film to be seen on the SSB and the surface of the corneocyte could therefore be seen in much greater detail. The sweat ducts were well marked and most presented an open lumen. The opening of the sweat ducts often projected above the surface (Fig. 5), a phenomenon which was more marked in the SSBs taken from the cornified area of the foot than in those taken from non-cornified areas. Large numbers of bacteria could be seen in SSBs taken from the occluded foot. These were predominantly associated with sweat ducts and were located within the lumen and on associated corneocytes (Fig. 5). Large colonies were also occasionally seen on the apparent ridges formed by the furrows in the skin surface (Fig. 6) (SSBs reveal the under surface of a layer within the stratum corneum).

The colonies shown by the SSB to be within the stratum corneum were much bigger than those seen on the surface of the skin. Colonies of more than 200 bacteria were common. All the bacteria seen were coccoidal in form. Pitting of the corneocyte surface was common (Figs 5 and 7) and appeared to be an indentation in the skin cell and not merely a cast in a superficial film.

*Unoccluded skin.* SSBs taken from the skin of the unoccluded foot revealed considerably fewer bacteria than did those taken from occluded foot. However, when these were present they were almost always seen in association with sweat ducts and occasional large colonies were seen.

## DISCUSSION

Bacteria were found not to favour any particular site on biopsies taken from the occluded foot, being seen in and around sweat ducts as well as on the open surface. Since the area of the foot that we have examined is hairless it is not possible, in this brief study, to comment on the significance of the infundibula of hair follicles as sites for the location of bacteria.

The reported values for the number of bacteria per colony on the stratum corneum vary from site to site and subject to subject. Somerville & Noble (1973) reported values which varied from sixteen on the female forearm to 5093 on the male forehead, and Holt (1971) gave values of 1000 on the male forearm and $10^5$ on the male abdomen. We also observed a great variation in colony size but most colonies contained very few bacteria, usually less than ten. The mean colony size was not calculated but it would have been much smaller (if expressed geometrically) than the values previously reported. Somerville & Noble (1973) and Holt (1971) calculated their values from total viable counts and colony formation on contact plates (Somerville & Noble) or from replica pads (Holt). Our observations have demonstrated that many bacteria will be inaccessible to a plate or pad applied to the surface although they may be eluted by a washing technique. This would tend to over-estimate the size of colonies. Similarly where small colonies are close together on the surface, as our observations show is often the case, then the contact plate or pad will not be able to resolve them and several small colonies will appear as a single colony. This will also tend to over-estimate colony size.

In general, all colonies whether within the stratum corneum or on the surface were flat and rarely more than two cells thick. For colonies within the stratum corneum this may be due to the restriction of vertical expansion imposed by the proximity of the layers of corneocytes. Lateral development of colonies may also be encouraged by the direction from which nutrients are delivered. Both between cells and on the surface it is more likely that nutrients will be delivered to the edge of the colony rather than from below or above.

When interpreting the growth of bacteria under conditions of complete occlusion it must be remembered that this is a condition which rarely occurs in nature. It has been shown by King *et al.* (1978) that occlusion with plastic film will increase the carbon dioxide emission rate of the skin and these workers have proposed that this may influence the growth and physiology of micro-organisms on the surface. However, partial occlusion of varying degrees of completeness will occur at intertrigenous sites. Thus whilst our model might not hold true for the arid regions of the body it may indicate the nature of things in such regions as the axilla, groin and toewebs.

We observed pitting of the surface of corneocytes in several of the specimens which we examined. The position of the pits and their size and shape suggested that they may have been produced by bacteria.

In biopsies from erythrasma lesions, Montes *et al.* (1965) observed penetration of corneocytes by bacteria, with an associated breakdown in the cell membrane of the corneocytes, but they did not observe penetration of the corneocyte in specimens taken from normal skin. However, transmission electron micrographs of normal skin produced by Montes & Wilborn (1970) quite clearly show the deformation of stratum corneum cells in the neighbourhood of bacteria.

Pitting of the stratum corneum was also observed by Dawber, Marks & Swift (1972) on biopsies taken from subjects suffering from bullous ichthyosiform erythroderma, but the size, shape and distribution of the pits was such as to suggest that they were not of bacterial origin. The pitting which we have observed may be due to keratolytic action although the retention of surface detail of the corneocyte within some of the pits makes this unlikely. It may, alternatively, be due to van der Waals attraction between the bacteria and the corneocyte, resulting in a deformation of the surface of the corneocyte.

The hydration of the skin surface brought about by occlusion increased the number of bacteria seen associated with sweat ducts in SSBs. It would appear that hydration permits the proliferation of the small numbers of bacteria which are normally present around the lumen of the ducts. However, large numbers of bacteria were never seen within the ducts themselves but mostly attached to the corneocytes surrounding the duct. It is possible that the majority of bacteria multiplying within the duct itself are flushed out by sweat, whereas those situated between the cells of the stratum corneum which surround the duct are able to multiply undisturbed within the moist environment.

Our observations on the association of bacteria with sweat ducts conflict with those of Lovell (1945) and Montes & Wilborn (1970) who were unable to find bacteria in or around the ducts. However, O'Brien (1945) was able to show large numbers of bacteria within that part of the sweat duct which passes through the stratum corneum. O'Brien argued that the blockage of the terminal portion of sweat ducts was one of the major factors responsible for causing miliaria rubra. Hölzle & Kligman (1978) have also provided evidence which suggests a bacterial involvement in miliaria and have observed bacteria around and occasionally within sweat ducts. Whatever the role in miliaria of bacterial multiplication within the sweat ducts, the work of these authors, like ours, suggests that the sweat ducts carry a resident population of bacteria. Indeed it may be suggested that the sweat ducts play a role as a refuge and reservoir for bacteria comparable at least to that ascribed to the infundibula of hair follicles. Unlike Noble (1975) and Hölzle & Kligman (1978) who examined skin which had been occluded for 4 h and 4 days respectively, we did not find any preferential development of bacterial colonies around the orifices of sweat ducts.

The depth to which bacteria are capable of penetrating the stratum corneum cannot be precisely stated, but light microscopic studies on sections cut from SSBs showed bacteria present to a depth of at least six cell layers (the thickness of the SSB) within the stratum corneum. Montes & Wilborn (1970) also showed bacteria deep within what they called the 'desquamated' portion of the stratum corneum. Because of the disruption of the layers which had taken place it is not possible to calculate from their photomicrograph how many layers of corneocytes comprise this 'desquamated' portion.

McBride, Duncan & Knox (1978) have shown a correlation between numbers of corneocytes and bacteria removed from the forehead by scrubbing. These authors suggest that bacteria are located within the stratum corneum and that they may be principally *Propionibacterium spp.* Whilst this may be the case on the forehead our observation that coccoidal bacteria can reside several cell layers deep within the stratum corneum suggests that this site is not the exclusive province of obligate anaerobes.

In our study we have examined both occluded and unoccluded stratum corneum and have shown that the process of occlusion greatly increases the number of bacteria visible by scanning electron microscopy.

The combined processes of occlusion and scanning electron microscopy may serve to demonstrate the location of bacteria on other sites which normally have a low bacterial population.

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# Acne vulgaris: is the bacterial population size significant?

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## SUMMARY

The levels of *Propionibacterium acnes* (*P. acnes*) and members of the *Micrococcaceae* were enumerated in two separate studies. The first investigation on the foreheads of thirty-five mild and thirty-five moderate acne patients showed no significant difference in the bacterial populations of the two groups. The second investigation of twelve patients on 250 mg tetracycline twice daily for 3 months showed no significant difference compared to pre-treatment data in the bacterial population during the successful treatment period.

The data indicate that greater numbers of bacteria are not associated with increasing severity of acne and that the effectiveness of oral tetracycline in treating the disease can not be explained by a reduction in the number of viable bacteria.

There have been several reports indicating that the successful treatment of acne with 1000 mg daily oral tetracycline decreases the population of *P. acnes* (Goltz & Kjartansson, 1966; Mills, Marples & Kligman, 1972). Cunliffe *et al.* (1973) found that a significant satisfactory clinical response but no decrease in the organisms within the pilosebaceous duct was obtained following treatment with oral tetracycline hydrochloride at 250 mg daily for 3 months. Therefore, it is possible that there may not be a direct relationship between the size of the bacterial population and the severity of acne.

The purpose of this study was to investigate the relationship between the size of the bacterial population and the extent of acne on the face using a surface sampling technique. Two studies were undertaken. The first compared the microbial populations of patients with mild and moderate acne and the second investigated the response of the microbial population to low dose tetracycline therapy.

## MATERIALS AND METHODS

The degree of acne was graded on a simple scoring system. This technique has been used in the department for 5 years. It is a 0–11 scale; zero representing no acne and subjects with a score of 1–3 have mild acne, 4–7 moderate acne and 8–11 severe acne. Assessment was made under excellent lighting conditions, the skin palpated as well as inspected. It is a subjective assessment, simple, quick to per-

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form and reproducible; it accounts for inflamed and non-inflamed lesions without counting their number. Concurrence of scoring between trained observers is high ($r = 0{\cdot}85$) (Cunliffe & Gould, unpublished observations).

### *Patients*

The patients were aged 18–20 years. They received no oral or topical therapy for at least 1 month before treatment. The investigations were performed during the autumn and winter to eliminate the variable effect of ultraviolet radiation on acne.

### *Tetracycline treatment schedule*

Patients were given 250 mg oxytetracycline orally twice daily. The tablets were taken half an hour before food and not with milk, and the treatment was continued for 3 months.

### *Sampling methods*

Bacteria were sampled using the 'scrub' technique (Williamson & Kligman, 1965). An area of involved skin on the face of each patient was selected for sampling prior to treatment and all subsequent samples were taken from the same site.

### *Bacteriological methods*

Colony forming units were determined by plating out tenfold serial dilutions of the wash fluid onto the appropriate recovery media. *Propionibacterium acnes* colonies were counted after 7 days anaerobic incubation at 37°C on Reinforced Clostridial medium (Oxoid Ltd). Members of the family *Micrococcaceae* were estimated after 48 h aerobic incubation at 37°C on heated blood agar (Oxoid Ltd). Bacterial numbers were expressed as $\log_{10}$ colony forming units $cm^{-2}$ skin.

## RESULTS

A comparison of the bacteriology of mild and moderate acne skin showed no significant difference in the number of microorganisms isolated either for *P. acnes* or the *Micrococcaceae* (Table 1).

The effects of oral tetracycline on the bacteriology and acne grade of twelve patients are given in Figs 1 and 2. A decrease in the number of *P. acnes* after the fourth week of treatment (Fig. 1) did not

TABLE 1. The enumeration of *Propionibacteria* and members of the *Micrococcacae* from patients with mild and moderate acne on the face

| | Mild acne | Moderate acne |
|---|---|---|
| Number of patients | 35 | 35 |
| Age (years) | 18–20 | 18–20 |
| Mean $\log_{10}$ *P. acnes* ($cm^{-2}$) ($P = 0{\cdot}2$)* | $5{\cdot}03 \pm 0{\cdot}19$ | $5{\cdot}35 \pm 0{\cdot}14$ |
| Mean $\log_{10}$ *Micrococcaceae* ($cm^{-2}$) ($P = 0{\cdot}1$)* | $4{\cdot}41 \pm 0{\cdot}17$ | $4{\cdot}77 \pm 0{\cdot}11$ |

* Bacteria were sampled using the scrub method of Williamson & Kligman (1965). The data were analysed using the Student's '*t*'-test (± s.e. mean).

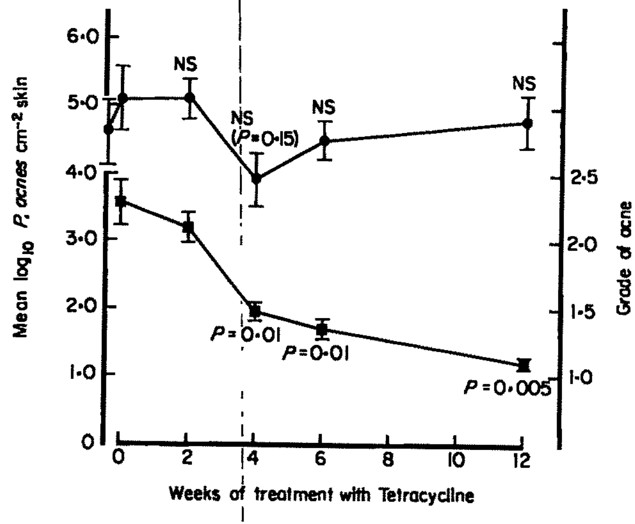

FIGURE 1. The effect of oral tetracycline therapy (250 mg twice daily) on the level of *P. acnes* and the mean acne grade of twelve patients.

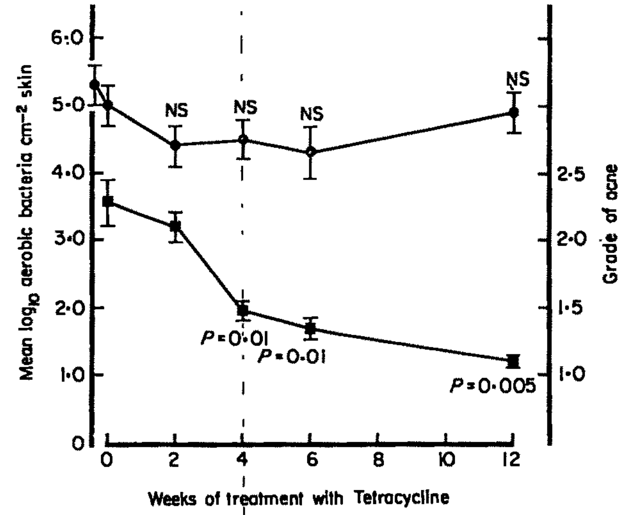

FIGURE 2. The effect of oral tetracycline therapy (250 mg twice daily) on the aerobic microflora and the mean acne grade of twelve patients.

approach the 5% significance level using the Student's '*t*'-test analysis. Throughout 3 months of treatment there was no significant decrease in the population of either *P. acnes* or the *Micrococcaceae.*

However, a significant decrease in the acne grade of these patients occurred after 4 weeks therapy. This improvement in the condition was maintained for the remainder of the study.

## DISCUSSION

The results of both studies clearly indicate that a relationship could not be established between the size of the microbial population and the severity of acne. This was demonstrated by a comparison of untreated mild and moderate acne patients and also in patients showing a clinical improvement while undergoing tetracycline therapy.

Although Aly, Maibach & Bloom (1978) estimated that the scrub method of Williamson & Kligman (1965) removed approximately 19·5% of the resident anaerobes in two minute washes, Leyden *et al.* (1975a) succeeded in demonstrating age related changes in the bacterial population of the human face using this technique. Thus, the sampling method used in this study can detect significant changes in the size of the bacterial population. Furthermore, Leyden *et al.* (1975b) found that patients with acne and normal subjects over the age of 21 showed no difference in the levels of *P. acnes*.

The data obtained from patients undergoing tetracycline therapy in this study contrast with those of Goltz & Kjartansson (1966) and Mills *et al.* (1972), but these investigators employed dose equivalents of tetracycline hydrochloride equal to or greater than 1000 mg daily. This difference in results may be explained by the dose of tetracycline used. A high dose inhibits the growth of bacteria on the skin whilst a dose of 500 mg tetracycline per day is insufficient to cause inhibition but is capable of reducing the acne grade.

Questions therefore arise as to the role of microorganisms in acne and to the mode of action of tetracycline in the treatment of acne. Since tetracycline in low doses does not reduce the bacterial population other explanations for its action should be considered. It is possible that tetracycline may exert its effect by a hitherto unknown mechanism which is unrelated to the skin microflora. Lee & Shalita (1978) reported that lipase was the active cytotoxin secreted by *P. acnes* and that tetracycline and erythromycin inhibited chemotaxis independently of any effect on lipase. It is therefore possible that tetracycline could affect the inflammatory response in acne. However, tetracycline has also been shown directly to inhibit *P. acnes* lipase (Hassing, 1971). Furthermore, it is possible that tetracycline could inhibit the production of bacterial lipases or other exoenzymes. Indeed tetracycline may act as a bacteriostatic agent or could impair bacterial functions which are related to the causation of acne. Thus microorganisms may not express their role in acne by an increase in number but possibly by expressing different characters with changes such as pH, Eh, $pCO_2$, oxygen tension and nutrients within the micro-environment of the pilosebaceous duct.

## ACKNOWLEDGMENTS

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# Proliferative response to fluid injection in psoriatic and normal human epidermis

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## SUMMARY

Feulgen-DNA cytophotometry revealed that human epidermal cell proliferation is regulated at a pre-DNA synthetic phase and can be perturbed by mild trauma. Although uninvolved psoriatic skin appears clinically normal, its hypersensitivity indicates abnormal proliferative behaviour and may serve as a marker of latent disease.

Despite considerable effort, the pathogenesis of psoriasis is not yet clear; however, the observed benign epidermal hyperplasia does indicate alteration in cell-cycle controls. Previous autoradiographic studies have shown that, in psoriasis, epidermal cell proliferation can be drastically elevated mechanically by repeated tape-stripping (Braun-Falco, Christophers & Kurban, 1967; Christophers & Braun-Falco, 1970) or pharmacologically with the $\beta$-adrenergic blocker, propranolol (Wiley & Weinstein, 1977). In the present investigation, we demonstrate that just needle puncture and intradermal fluid injection is sufficient trauma to perturb the cytokinetics of the psoriatic epidermis. By using Feulgen-DNA cytophotometry to monitor this response not only do we eliminate the need for radioisotopes, but also gain some insight into the principal cell cycle regulatory phase in the human epidermis.

The purpose and nature of this investigation were explained to each subject, and informed consent obtained. Using a 23-gauge 3/8 inch needle, 0·1 ml of physiological saline was injected into the edge of an active psoriatic lesion and a clinically normal-appearing area approximately 2·5 cm away. The sites were marked with a felt-tipped pen to aid in re-identification. Thirty hours later, these sites, representing injected involved and injected uninvolved psoritic epidermis respectively, were biopsied under local anaesthesia using a 4 mm punch. At this time, additional samples representing non-injected involved and non-injected uninvolved psoriatic skin were obtained from sites approximately 1 cm away from their injected counterpart. Specimens were also obtained at similar sites and times from age and sex matched normal control subjects with no significant dermatological problems. The biopsies were fixed in 10% neutral buffered formalin and processed in the routine manner. Eight micron sections were stained by a modified Feulgen procedure and single cell DNA contents were measured using a Vickers M-85 microspectrophotometer as previously described (Grove, Anderson & Smith, 1976; Grove, Lavker & Kligman, 1978; Grove & Smith, 1976). A total of 100 interphase cells

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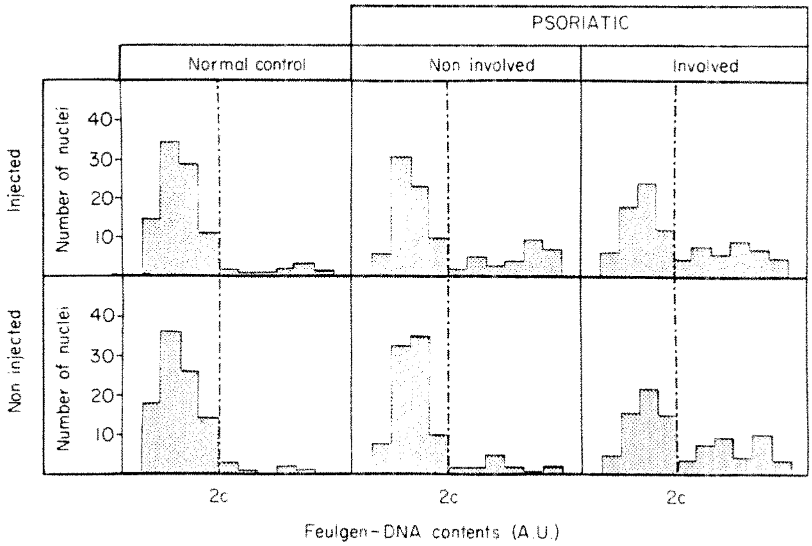

FIGURE 1. Representative profiles of nuclear DNA contents obtained by using a Vickers M85 microspectrophotometer to measure the integrated density of 100 individual Feulgen-stained epidermal cells. Broken line represents an arbitrary diploid/hyperdiploid boundary based on twenty-five measurements of non-proliferative lymphocytes in the subepidermal infiltrate.

TABLE 1. Percentage of germinative cells with >2C Feulgen-DNA contents

| | | | | Psoriatic | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normal | | | Uninvolved | | | Involved | | |
| | Non | Inj | Δ% | Non | Inj | Δ% | Non | Inj | Δ% |
| | 8 | 12 | 50 | 12 | 29 | 141 | 38 | 35 | −7 |
| | 6 | 11 | 83 | 16 | 36 | 125 | 26 | 39 | 8 |
| | 9 | 13 | 44 | 17 | 35 | 106 | 42 | 40 | −5 |
| | 7 | 10 | 43 | 14 | 33 | 136 | 33 | 36 | 9 |
| Mean | 7·5 | 11·5 | 55·0 | 14·7 | 33·2 | 127·0 | 37·3 | 37·5 | 1·3 |
| S.e. mean | 0·6 | 0·6 | 9·5 | 1·1 | 1·5 | 7·8 | 1·9 | 1·2 | 4·2 |

of the proliferative compartment were measured in triplicate slides from each skin sample. Lymphocytes of the sub-epidermal infiltrate were used as an internal standard of reference for the 2C or diploid amount of Feulgen-DNA.

Figure 1 shows representative histograms of Feulgen-DNA contents of individual germinative cell nuclei for each group measured in this study. To facilitate statistical comparisons of the groups, dotted lines were included on the histograms using the 2C DNA mode of the non-proliferative lymphocyte population as a reference. It was reasoned that the number of cells with DNA contents exceeding the 2C level gave an indication of the relative proliferative activity since it is these cells which are synthesizing DNA and preparing to divide.

As shown in Table 1 a comparison of the non-injected sites reveals that the involved psoriatic epidermis has the highest percentage of such hyperdiploid cells. In addition the relative proliferative activity of the uninvolved psoriatic epidermis is slightly elevated in comparison to normal controls. A comparison of the effects of intradermal fluid injection indicates a differential sensitivity to this type of mild trauma. In the case of involved psoriatic skin, no differences could be seen between the injected and non-injected lesions. This finding supports the contention that nearly all the cells in the germinative layers of an active lesion are cycling at their maximal rates (Weinstein & Frost, 1968, 1969). On the other hand the number of hyperdiploid cells is strikingly increased in the injected sites of uninvolved psoriatic skin. This is also true, but to a lesser extent, for non-psoriatic controls. Thus, although the uninvolved psoriatic skin appears clinically normal, its proliferative behaviour is abnormal. Hopefully, this hypersensitivity to trauma whether by fluid injection or more drastic mechanical and pharmacological means may be of potential clinical value as a marker for latent disease.

It should be emphasized that by using Feulgen-DNA cytophotometry not only has the need for radioisotopes been eliminated but some information on the principal phase of cell cycle control in the human epidermis has been obtained. Previous studies (Gelfant & Candelas, 1972; Goodwin, Hamilton & Fry, 1974; Gelfant, 1976) have suggested on the basis of indirect evidence that a post-DNA synthetic event may be important in regulating the proliferation of this population. If this were true, the 4C or tetraploid mode containing arrested $G_2$ phase cells should predominate in those instances where proliferative activity is low. The present results clearly show that this is not true and provide direct evidence that a pre-DNA synthetic event is the principal site of regulation. At the present time, it is not known whether human epidermal cells have a $G_1$ phase of variable duration which can be shortened drastically upon stimulation or whether they sojourn in a discrete $G_0$ non-cycling state from which they can be provoked to revert to a cycling state (Baserga, 1976; Grove, 1979). Unfortunately, since both $G_1$ and $G_0$ are pre-DNA synthetic phases, they cannot be resolved on the basis of DNA contents.

It should be emphasized that the extreme sensitivity of the uninvolved psoriatic epidermis to mild trauma demonstrated in this study has an impact on the tactics one should use in cell cycle studies of this disease. The injection of tritiated thymidine which is a requisite of any autoradiographic assessment of proliferative activity *in vivo* is the equivalent of the mild trauma utilized in the present study. Although it is unlikely that short-term studies of 1 or 2 h would pose a problem, previous assessments of growth fraction which required multiple injections of methotrexate or tritiated thymidine (Weinstein, 1975) must be viewed with great caution. Clearly, additional studies will be required before a complete understanding of the abnormal cell kinetics in psoriasis are fully understood.

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# Unscheduled DNA synthesis in psoriatic skin after ultraviolet irradiation and the effects of a combined treatment with 8-methoxypsoralen and longwave ultraviolet radiation: a clinical study*

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## SUMMARY

Biopsies were taken from psoriatic patients undergoing photochemotherapy and then studied by autoradiography to investigate possible unscheduled DNA synthesis (UDS) following 8-MOP photodamage in epidermal cells. No sparse labelling was found in seventy samples taken from twenty-four different patients during PUVA therapy, indicating a lack of UDS. In contrast, UDS was observed in three patients irradiated with an all-spectrum UV lamp, as a test for DNA damage and excision repair activity. The incorporation of $^3$HTdR in these keratinocytes which were not undergoing S-phase was not affected by the dose of UV-A received during different stages of PUVA treatment.

The results are discussed in terms of repair of the induced photodamage at the level of DNA, and in relation to a possible risk of this treatment when it is given over a long period of time.

Photochemotherapy using psoralen (P) and high intensity long wavelength ultraviolet irradiation (UV-A), i.e. PUVA, is widely used with great effect in several skin disorders, in particular psoriasis (Parrish *et al.*, 1974; Swanbeck *et al.*, 1975; Wolff *et al.*, 1976; Parrish, 1977). The success of this therapy is due to a decrease in epidermal turnover which is probably the result of a direct effect on epidermal DNA replication (Walter *et al.*, 1973; Epstein & Fukuyama, 1975). Such effects result from the formation, in the DNA molecule, of monofunctional and bifunctional photoadducts leading to interstrand cross-links with photosensitizing psoralens in the presence of UV-A (Musajo, Bordin & Bevilacqua, 1967; Cole, 1971).

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UV-induced pyrimidine dimers formed by UV-B irradiation can be repaired by an excision process which is an essential property of normal cells (Epstein, Fukuyama & Epstein, 1969; Pathak, Krämer & Gungerich, 1970). Unscheduled DNA synthesis (UDS), which represents the incorporation of new bases into the damaged strands of DNA after the excision, was found in irradiated skin (254 nm) following labelling with tritiated thymidine ($^3$HTdR). After autoradiography there was a sparse labelling throughout the epidermis and in the upper part of the dermis.

Some experimental data concerning the excision-repair mechanisms for psoralen-photodamaged DNA have been reported for unicellular organisms (Igali, 1972; Chandra, Kraft & Wacker, 1971; Cole, 1973) and human fibroblast culture (Baden *et al.*, 1972; Carter, 1975). Baden *et al.* (1972) compared the effects of two procedures: 8-methoxypsoralen (8-MOP) plus UV-A and 254 nm irradiation on DNA excision repair synthesis in fibroblast cultures. After treatment with 8-MOP plus UV-A, tritiated thymidine was incorporated into DNA at a slower rate than after 254 nm irradiation. Recently, Coppey, Averbeck & Moreno (1979) demonstrated that the repair capacity of human fibroblast cultures was very limited after 8-MOP (a bifunctional furocoumarin) plus UV-A, in contrast to the repair of the monofunctional furocoumarin-induced damage (Angelicin).

However, information is very scarce concerning keratinocytes, which are the target cells for the PUVA therapy (Swanbeck *et al.*, 1976; Gschnait, Brenner & Wolff, 1978).

Thus, the following experiments were performed in order to study the excision repair process detected in epidermal cells, after long term PUVA therapy in psoriatic patients, under clinical conditions.

Parallel studies were performed on psoriatic skin following irradiation with shorter UV light.

## MATERIALS AND METHODS

Eighty-nine cutaneous biopsies of psoriatic patients undergoing PUVA therapy in the Department of Dermatology were performed before and during treatment. These tissue specimens were studied for $^3$HTdR uptake. However, only the results from twenty-four patients were chosen to be reported here because in these patients several biopsies during the therapy protocol allowed a complete study of any change during the therapy.

### *PUVA irradiations and biopsies*

Twenty-four patients with widespread psoriasis were investigated: fifteen men and nine women, ranging from 8 to 69 years of age. Two hours after ingestion of methoxsalen (Meladinine, 10 mg/20 kg body weight), the patient was irradiated in a hexagonal cabin giving total body irradiation. This light box contained vertical rows of UV-A lamps (Philips TL/09) which had a continuous spectrum of high intensity UV irradiation (320–400 nm) with a peak emission at 365 nm. The time of initial exposure depended on the skin type. The initial UV-A dose received by the patient at a distance of 50 cm was about 1·5–2 J/cm$^2$ and was gradually increased to a maximum of 6–7 J/cm$^2$ for a single session.

2·5 mm punch biopsies were performed at several stages:

(1) before PUVA treatment—on skin unexposed to sunlight; on uninvolved skin; and on psoriatic plaques (thirty-seven biopsies).

(2) during different stages of the treatment programme. Thus, biopsies were separated into five groups. Group A included the biopsies performed after the first exposure (fifteen cases). Groups B to E (fifty-five cases) included the biopsies performed after a number of exposures ranging from the

TABLE 1. The number of skin biopsies taken after varying periods of PUVA treatment and different intervals after the exposure

| Group | | A | B | C | D | E |
|---|---|---|---|---|---|---|
| Number of PUVA exposures | | 1 | 2–10 | 11–20 | 21–50 | 51–103 |
| (cumulative dose of energy—J/cm$^2$) | | (3) | (22) | (55) | (150) | (480) |
| Number of skin biopsies | ½ h after exposure | 15 | 10 | 15 | 8 | 10 |
| | 1–2 h after exposure | | 1 | 1 | | 5 |
| | 24 h after exposure | | 1 | 1 | 2 | 1 |

second to 103rd exposure, which corresponded to a cumulative UV-A dose of 3–480 J/cm$^2$ (Table 1). The results of the biopsies for each patient were distributed to three or more of the groups depending on the time at which the biopsy was taken after the initial treatment.

Biopsies of an improved psoriatic lesion without scales were taken at varying intervals after the session of ultraviolet irradiation: usually in the first half-hour (fifty-eight times), after 1–2 h (seven times) and 24 h (five times). At the same time, a control biopsy was taken from a small protected (thus non-irradiated) area of skin (thirty-seven times). In total, seventy biopsies taken during PUVA therapy and thirty-seven non-irradiated controls were analysed. These experiments are summarized in Table 1.

As controls, three psoriatic men were irradiated with an all-spectrum UV lamp, before PUVA treatment (Patients 1, 2, 3).

In these patients, a 10 cm$^2$ surface of the back was irradiated by a 600 W hot quartz lamp which emitted in the short, middle and long UV light range. At a target distance of 15 cm, the energy delivered at the skin was approximately 10 mW/cm$^2$. The exposure time was 5 min in Patients 1 and 2 and 6 min in Patient 3. Punch biopsies were obtained immediately after exposure in each patient and again after 30 min in Patient 2. Two additional biopsies were performed after 30 min and 24 h in patient 3. Non-irradiated control specimens were also biopsied in each patient.

### *$^3$HTdR labelling, embedding and sectioning*

Skin samples were cut into 1 mm$^3$ pieces and placed in 1 ml of Eagle's medium supplemented with 20% fetal calf serum and containing 10 or 20 $\mu$Ci/ml of methyl $^3$H thymidine (specific activity: 25 Ci/mM; CEA, Saclay). Incubation was made at 37°C for 3 h, or longer for some biopsies (6 h). The fragments were then washed three times in a solution of phosphate buffer, fixed for 1 h at 4°C in 2·5% glutaraldehyde made up in 0·1 M phosphate buffer and then rinsed in the same buffer. They were then post-fixed in 1% osmium tetroxide for 1 h, dehydrated in alcohol and embedded in Epon 812. The blocks were cut into sections 1 $\mu$m thick and prepared for autoradiography.

### *Autoradiography*

Tissue sections were coated with diluted emulsion (Ilford K5). The autoradiographs were exposed at 4°C for 2–4 weeks. The slides were then developed for 6 min in D19 developer, fixed, washed and finally stained with toluidine blue.

### *Microscopic analysis*

Autoradiographs of histological specimens were examined with a Zeiss microscope using a ×100 objective and a micrometer in a × 10 eyepiece. To facilitate counting, a video system consisting of a camera and a 24 cm TV monitor was used.

### *Statistical analysis of grain count*

To determine the background activity, the mean value ($\bar{X}$) of grains/nucleus for samples of non-irradiated skin obtained before PUVA treatment was calculated. About 200 nuclei were counted on each slide. The mean value ($\bar{X}$) followed a normal distribution. The 95% confidence limit was calculated: $\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$ ($P < 0{\cdot}05$, S = standard deviation and N = total number of nuclei counted). The percentage of cells with a grain count greater than $\bar{X} + 1{\cdot}96\ S/\sqrt{N}$ is less than 16% when only the background population was present (i.e. Gaussian distribution). If other grain populations were present with a mean value ($\bar{X}'$) greater than $\bar{X}$, the percentage of nuclei with a grain count in excess of $\bar{X} + 1{\cdot}96\ S/\sqrt{N}$ would be greater than 16%.

$^3$HTdR uptake analysis revealed three classes of labelled cells: cells with a very low number of grains corresponding to background, sparsely labelled cells corresponding to cells undergoing UDS and heavily labelled cells corresponding to cells undergoing semi-conservative DNA synthesis (>25 grains/nucleus). Only cells with low labelling (i.e. background and UDS) were considered in this study.

## RESULTS

For each slide, the number of cells in the entire epidermis was counted (mean 200 cells). One hundred and forty-four slides were examined: thirty-seven before treatment, seventy during PUVA therapy and thirty-seven non-irradiated controls (skin protected during irradiation).

The counting of cells with very light labelling in the range of the background activity was determined using the thirty-seven biopsies before treatment: i.e. nineteen biopsies of uninvolved skin ($\bar{X}$ = 1·19 ± 0·04) and eighteen biopsies of psoriatic skin ($\bar{X}$ = 0·8 ± 0·05) (Table 2).

TABLE 2. The mean value of grains/nucleus: $\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$, for the control biopsies and for groups A to E, ½ h after PUVA exposure. Cells undergoing S phase were not counted. Incubation time in $^3$HTdR: 3 h

| | Uninvolved skin<br>N = 3868 | Psoriatic skin<br>N = 3397 |
|---|---|---|
| Before PUVA | 1·19 ± 0·04 | 0·80 ± 0·05 |
| During PUVA | Non-irradiated controls<br>N = 5510 | PUVA exposed skin<br>N = 8702 |
| Group A | 0·69 ± 0·05 | 0·68 ± 0·04 |
| B | 0·83 ± 0·07 | 0·57 ± 0·07 |
| C | 1·16 ± 0·08 | 0·98 ± 0·05 |
| D | 0·66 ± 0·09 | 0·64 ± 0·10 |
| E | 0·60 ± 0·07 | 0·59 ± 0·05 |
| For all the samples | 0·80 ± 0·03 | 0·75 ± 0·02 |

N = number of nuclei counted.

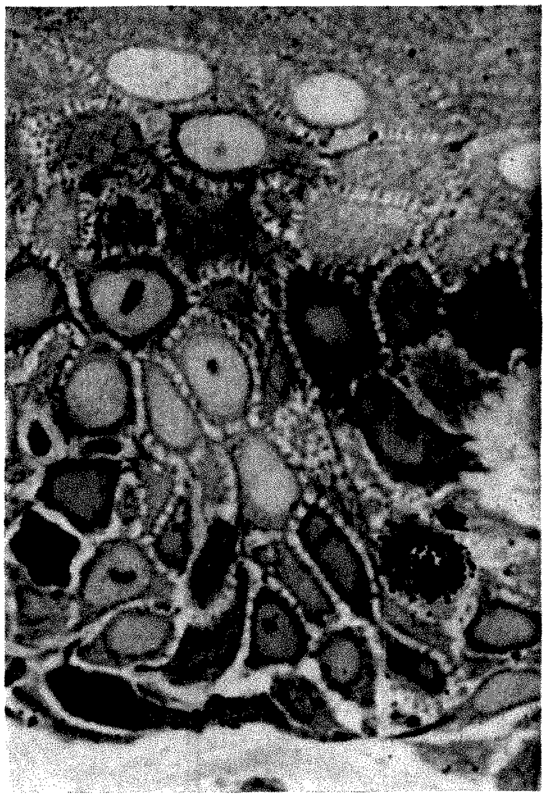

FIGURE 1. Autoradiogram of skin taken ½ h after ninety-fourth PUVA exposure. There is no sparse labelling in the epidermis. Arrow indicates a cell in semi-conservative DNA synthesis (Case AT, Table 3) (× 800).

*Analysis of biopsies performed after photochemotherapy with* 8-*MOP plus UV-A*

In PUVA treated patients, regardless of the dose of energy received in a single session and regardless of the number of exposures, the mean number of grains/nucleus was the same as that for the non-irradiated controls (skin protected during PUVA), i.e. $<1{\cdot}5$ grains/nucleus. This value did not change after the first exposure (sample A) and after a varying number of PUVA exposures (samples B, C, D, E). The results are summarized in Table 2. Figure 1 is an example of such a skin specimen taken after PUVA exposure.

When considering the five groups together (A–E) the mean number of grains/nucleus for irradiated skin ($X = 0{\cdot}75 \pm 0{\cdot}02$) was not significantly different from the mean number of grains/nucleus obtained for non-irradiated controls performed at the same time ($\bar{X} = 0{\cdot}80 \pm 0{\cdot}03$).

Table 3 shows five examples of the number of grains/nucleus against time for patients biopsied in different groups.

For the samples taken at various times after irradiation it was found that the mean number of grains/nucleus was not significantly different after the first half-hour, 1 h and 24 h after PUVA exposure. This value was always $<1{\cdot}5$ grains/nucleus (Table 4).

For some specimens, the $^3$HTdR incubation time was increased to 5 h in an attempt to detect any

TABLE 3. The mean value of grains/nucleus ($\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$) for five examples of patients biopsied in different groups, ½ h after PUVA exposure

| Patient code | Before PUVA | During PUVA Group A | B | C | D | E |
|---|---|---|---|---|---|---|
| W | u:1·20 ± 0·12<br>i:0·62 ± 0·12 | $t_1$:0·63 ± 0·13<br>(0·34 ± 0·10) | $t_3$:0·36 ± 0·11<br>(0·51 ± 0·12) | | $t_{22}$:1·34 ± 0·30 | |
| AD | u:0·36 ± 0·11<br>i:0·92 ± 0·29 | $t_1$:0·48 ± 0·31 | | $t_{14}$:0·10 ± 0·06 | $t_{40}$:0·27 ± 0·12 | |
| AH | u:0·73 ± 0·12<br>i:0·30 ± 0·11 | $t_1$:0·23 ± 0·12<br>(0·33 ± 0·15) | $t_{10}$:0·12 ± 0·10 | | $t_{24}$:0·31 ± 0·10<br>(0·43 ± 0·10) | |
| AG | u:1·49 ± 0·19<br>i:0·94 ± 0·19 | $t_1$:0·59 ± 0·23<br>(0·58 ± 0·16) | t :0·40 ± 0·13<br>(0·72 ± 0·38) | | | $t_{68}$: 0·70 ± 0·13<br>(0·70 ± 0·13) |
| AT | u:1·33 ± 0·15<br>i:0·67 ± 0·14 | $t_1$:0·48 ± 0·11 | | | | $t_{67}$:0·66 ± 0·14<br>(0·65 ± 0·10)<br>$t_{94}$:0·35 ± 0·10 |

t = number of exposure.
u = biopsy on uninvolved skin.
i = biopsy on psoriatic skin.
Mean value of grains/nucleus on non-irradiated controls taken at the same time in parentheses.

TABLE 4. The mean values of grains/nucleus $\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$ for biopsies taken after various intervals after PUVA exposure. Incubation time in $^3$HTdR: 3 h. Number of nuclei counted (N) is for all of the samples in groups A to E, because there was no significant difference in grain count between these groups (see Table 1)

| Time after exposure (h) | Non-irradiated control | PUVA exposed skin |
|---|---|---|
| ½ | 0·80 ± 0·03<br>N = 5510 | 0·75 ± 0·02<br>N = 8702 |
| 1 | 0·93 ± 0·07<br>N = 840 | 0·88 ± 0·07<br>N = 1366 |
| 24 | 1·30 ± 0·12<br>N = 387 | 0·69 ± 0·08<br>N = 709 |

TABLE 5. The effect of incubation time in $^3$HTdR on the mean value of grains/nucleus $\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$. Biopsies before treatment and ½ h after the irradiation were used. Number of nuclei counted (N) is for all of the samples in groups A to E, whether or not irradiated, because there was no significant difference in grain count between these groups (see Tables 1 and 2)

| Incubation time (h) | | $\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$ | N |
|---|---|---|---|
| 3 | before PUVA | $0{\cdot}91 \pm 0{\cdot}03$ | 6302 |
| | after PUVA | $0{\cdot}77 \pm 0{\cdot}02$ | 14,212 |
| 4 | after PUVA | $0{\cdot}98 \pm 0{\cdot}05$ | 1644 |
| 5 | after PUVA | $1{\cdot}78 \pm 0{\cdot}18$ | 262 |

sparse labelling which may have taken place. However, only a slight increase in the background activity ($\bar{X} = 1{\cdot}78 \pm 0{\cdot}18$) was observed (Table 5).

*Analysis of biopsies performed after irradiation with an all-spectrum UV lamp*

Sparsely labelled cells were found to be uniformly dispersed throughout the layers of the epidermis in biopsies taken from the three patients who were irradiated with an all-spectrum UV lamp (Fig. 2).

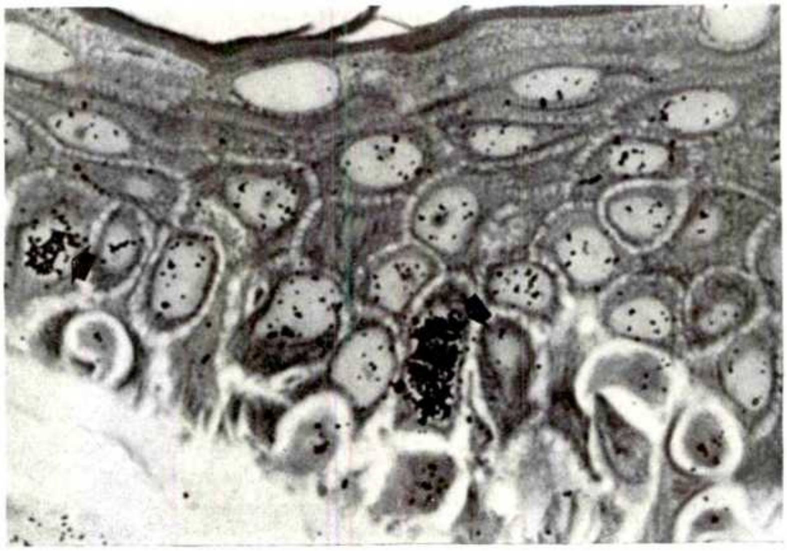

FIGURE 2. Autoradiogram of skin biopsied ½ h after irradiation with an all-spectrum UV lamp showing sparse labelling in all layers of the epidermis. This sparse labelling, corresponding to DNA repair synthesis, contrasts with heavy labelling of two basal cells (arrows) (Case 3, Table 5) (× 800).

TABLE 6. The mean value of grains/nucleus after irradiation with an all-spectrum UV lamp: $\bar{X}' \pm 1{\cdot}96\ S/\sqrt{N}$ compared with non-irradiated controls ($\bar{X} \pm 1{\cdot}96\ S/\sqrt{N}$)

| | Unexposed control skin | | Exposed skin | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Time biopsy after exposure | | | | | |
| | | | | | Immediately | | 30 min | | 24 h | |
| Patient | X | P | Exposure time (min) | Incubation time (h) | X′ | P | X′ | P | X′ | P |
| 1 | $1{\cdot}81 \pm 0{\cdot}21$<br>N = 214 | 14 | 5 | 6 | $14{\cdot}16 \pm 0{\cdot}96$<br>N = 282 | 96 | | | | |
| 2 | $0{\cdot}55 \pm 0{\cdot}12$<br>N = 174 | 14 | 5 | 3 | $5{\cdot}93 \pm 0{\cdot}57$<br>N = 346 | 80 | $5{\cdot}72 \pm 0{\cdot}41$<br>N = 461 | 82 | | |
| 3 | $0{\cdot}68 \pm 0{\cdot}15$<br>N = 136 | 17 | 6 | 3 | $9{\cdot}64 \pm 0{\cdot}5$<br>N = 429 | 94 | $9{\cdot}09 \pm 1{\cdot}03$<br>N = 161 | 85 | $4{\cdot}4 \pm 0{\cdot}44$<br>N = 396 | 76 |

P = percentage of cells with a mean number of grains greater than $\bar{X} + 1{\cdot}96\ S/\sqrt{N}$.
N = number of nuclei counted.

Table 6 summarizes the results of $^3$HTdR uptake immediately, 30 min and 24 h after irradiation with different doses of UV light and after labelling with $^3$HTdR for 3 or 6 h. In these slides, the population of cells undergoing UDS was characterized by two values: the mean number of grains/nucleus ($\bar{X}'$) was significantly different from $\bar{X}$ and the percentage of sparsely labelled cells (P) with a mean number of grains/nucleus in excess of $\bar{X} + 1{\cdot}96\ S/\sqrt{N}$ was much greater than 16%.

The incorporation of $^3$HTdR in the epidermis in all of these specimens was uniform. Sparse labelling was also observed in the cells of the upper part of the dermis i.e. fibroblasts, endothelial and perithelial cells, and in keratinocytes of the hair follicles.

## DISCUSSION

Several reports have shown that cells undergoing S-phase have a marked response to PUVA treatment. However, the effect of such treatment on non-S-keratinocytes has not been studied in detail.

Our experiments show that the energy received at the body surface and the duration of PUVA treatment do not modify the incorporation of $^3$H-thymidine in this population of epidermal cells. Thus, no significant difference was found between the mean number of grains/nucleus from control samples taken before PUVA (i.e. background activity) and samples obtained after one or several PUVA exposures, various times after each PUVA exposure and from skin covered during irradiation.

The biological effects of psoralen and UV-A irradiation in micro-organisms and in mammalian cells (Chandra *et al.*, 1973; Dall'Acqua *et al.*, 1974) are known and several authors have demonstrated that some furocoumarins produce DNA damage by the formation of mono- and bifunctional photoadducts. These adducts have been shown to occur in mammalian skin irradiated *in vivo* (Pathak & Krämer, 1969). Woodcock & Magnus (1976) have shown that, like UV-B alone, 8 MOP + UV-A produces sunburn cells in mouse epidermis. Hence, there is a similarity between sunburn cells and cells with DNA damage induced by PUVA treatment.

The induction of DNA repair synthesis in normal human fibroblasts has been reported after 8-MOP plus UV-A treatment, *in vitro*. Baden *et al.* (1972) have shown that after local application of labelled psoralen and UV-A irradiation, there was a progressive elimination of the photoadduct. The authors considered that the first stage in the repair mechanism of normal fibroblasts was the excision of the photoadduct, which is similar to that reported after DNA damage with 254 nm radiation. Using application of labelled psoralen and subsequent DNA extraction in guinea-pig skin, Chandra *et al.* (1973) demonstrated that DNA damage by photobinding of furocoumarins was repaired, but the repair of monofunctional adducts took place quicker than that of the bifunctional adducts.

In our experiments concerning psoriatic patients subjected to PUVA therapy, any sparse labelling indicative of DNA excision repair of the photodamage was not found. This finding may be taken to suggest that the degree of damage is so weak that the sensitivity of the autoradiographic technique may not be sufficient to demonstrate the DNA repair. The amount of photodamage induced by comparable doses of psoralen and UV-A on cells *in vitro* and on whole skin may be vastly different. Even in skin subjected to an irradiation of 7 J/cm$^2$ in a single session, no UDS was detected and different conditions of exposure to $^3$HTdR did not show any $^3$HTdR uptake. However, under the same experimental conditions, irradiation with an all-spectrum UV lamp produced a $^3$HTdR uptake representing UDS, the degree of which depended on the dose of UV light and the duration of $^3$HTdR labelling.

According to the experimental evidence, however, PUVA therapy induces DNA damage and the present study fails to show its repair by an excision mechanism, if UDS is accepted as being evidence of an excision mechanism.

If the damage is not repaired, then the structural changes will accumulate during PUVA therapy and therefore there may be a risk of mutation and transformation of cells. The programme of PUVA therapy, however, is not continuous and total clearing of the psoriasis is often obtained after twenty to thirty exposures. Epidermal turnover may progressively eliminate keratinocytes which contain damaged DNA. However, psoriasis generally presents as a chronic condition and is not halted indefinitely when PUVA therapy is terminated. To maintain positive results often requires repeated exposures over a long period of time to prevent new lesions. Repeated but weak DNA damage in keratinocytes over a long period of time raises the question of the possible cytogenetic effects of the treatment.

Actinic keratosis is a premalignant condition which appears in people exposed to the sun for long periods and it has been shown that leukocytes (Lambert, Ringborg & Swanbeck, 1976) and fibroblasts (Sbano *et al.*, 1978) from such patients had a reduced DNA excision repair mechanism whilst the activity of this mechanism was increased in exposed but normal skin. It has been suggested that there is a relationship between the alteration of DNA repair and the development of actinic cancer (Epstein, Fukuyama & Epstein, 1971).

Otherwise, the existence of increased sister chromatid exchange (SCE) rates may reflect damage and repair in mammalian cells (Latt, 1974). An increased SCE count in leukocytes after treatment with 8-MOP and UV-A *in vitro* has been demonstrated (Carter, Wolff & Schnedl, 1976). However, this increase in SCE in the lymphocytes of PUVA treated patients has not been observed (Mourelatos *et al.*, 1977; Wolff-Schreiner *et al.*, 1977; Lambert *et al.*, 1978). The lymphocyte population *in vivo*, as in our study of keratinocytes of whole skin *in vivo*, does not receive the same kind of exposure to 8-MOP and UV-A during a PUVA treatment as cells treated *in vitro*.

The possibilities of harmful cytogenetic effects of PUVA treatment have been studied mainly in lymphocytes (Swanbeck *et al.*, 1975) but very little is known about the effects on keratinocytes which are the target cells directly involved in the therapy. In contrast to our results, Swanbeck *et al.* (1976) using a model similar to ours, demonstrated that sparse labelling in the epidermis was the same after UV-B or PUVA. However, this work was presented as only preliminary experiments. Gschnait *et al.* (1978) demonstrated that DNA repair after UV-A was diminished in PUVA-treated patients. The authors suggested that the tan induced by PUVA therapy had a photo-protective effect against the DNA damage produced by UV light. Hence, it may be that, as a result of the protective role of the tan, there is less DNA damage to be repaired.

Within the limitations of the technique used, our experiments have failed to detect sparse labelling in the epidermis after PUVA therapy. This lack may signify the low rate or absence of repair activity in the target cells of the epidermis, probably due to a very little photodamage in clinical PUVA therapy under our conditions of irradiation.

The present study was directed at the excision repair mechanism only, but it is possible that the repair of photodamage, and particularly interstrand crosslinks, involves other repair mechanisms requiring further study. Furthermore, the effect on the DNA repair of other furocoumarins which produce only monofunctional photoadducts should be studied.

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## ADDENDUM

Since this article was submitted for publication, it has been drawn to our attention that Simone Bishop has obtained similar results to ours. She has detected UDS in skin specimens which was due to a small amount of UV-B emitted by the Sylvania tubes used in her study.

*British Journal of Dermatology* (1980) **102**, 297.

# The use of the Psorilux 3050A machine in the management of psoriasis of the hands and feet

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## SUMMARY

Eleven patients with severe psoriasis involving the palms and soles have been treated using a high-energy source of UV-A and UV-B with no additional topical or systemic photosensitizing drug. The results showed marginal benefit of UV-A alone over conventional topical tar therapy. The high energy output of the machine makes it essential that personnel supervising therapy are adequately protected.

In the past 5 years the use of psoralens administered either orally or topically followed by irradiation with long wave ultraviolet in the 315–400 nm range (UV-A) has become a highly popular method of managing patients with psoriasis. The numbers of patients treated by this method both in Europe and in North America are considerable and in general excellent results are claimed (Parrish *et al.*, 1974; Wolf *et al.*, 1976; Swanbeck *et al.*, 1975; Melski *et al.*, 1977; Roenigk *et al.*, 1979). However, a current British MRC funded study suggests that in the clearing phase of psoriasis there is no significantly increased clearing time by comparison with dithranol-treated patients although PUVA-maintained remissions resulted in longer lesion-free intervals than discontinuation of topical therapy after clearance has been achieved (Rogers *et al.*, 1979).

A recently reported survey of a large population of PUVA treated patients in North America shows at present a slight increase in the number of squamous carcinomata and a reversal of the normal ratio of basal cell carcinoma to squamous carcinoma. This ratio is normally 2:1 (Stern *et al.*, 1979). There is as yet no information on a changing incidence of malignant melanoma in PUVA treated patients.

Because of this concern over cumulative psoralen and UV-A exposure there is currently a renewed interest in the use of UV as a sensitizer with topical tar (Petrozzi *et al.*, 1978). An alternative approach which offers the benefit of a clean, odour free method of therapy is the use of high energy UV-A without the oral ingestion or topical applications of a photosensitizing drug. This approach has been facilitated by the development of the Psorilux 3050A apparatus (Siemens Ltd).

We have recently had the opportunity of assessing this machine in the management of eleven patients with psoriasis of the hands and feet.

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## MATERIAL AND METHODS

The Psorilux 3050A is a relatively small portable apparatus mounted on a stand. The lamp source measures 680 mm by 760 mm. Two high pressure mercury vapour lamps emit high intensity UV in the range 250–400 nm, and a filter (QLG F34) is provided which blocks all wavelengths below 320 nm. Thus UV-A+B can be provided without the filter and UV-A alone after insertion of the filter. Spectral irradiance curves obtained measured at 40 cm from the tube using a J221 Blackray meter show readings of 21·6 mW/cm$^2$ without the filter (300–400 nm) and 15 mW/cm$^2$ with the filter (220–300 nm) in position. This energy output is high by comparison with other commercially available PUVA machines for limited body areas. The energy output is such that the maximum daily permitted dose of UV can easily be exceeded by personnel operating the machine (WHO Environmental Health Criteria for Irradiation, 1978). This dose is exceeded if the operator stands in the direct line of the lamp for only 6 s, and therefore screening of the apparatus with cloth screens (standard hospital bed screens) and the use of cotton gloves and goggles is essential. With these precautions, if the operator does not stand in the direct line of the beam and keeps 1 m or more from the light source, permitted exposure is 60 min/day.

The control panel of the lamp is mounted at the rear of the light source and the precautions against excessive UV exposure make operation of the controls difficult. The relocation of these controls on a separate panel to be kept outside the screen area is recommended. After a 20 s warm-up after switching on the lamp, a 'ready light' appears on the control panel and this also should be relocated. The range of adjustment of the lamp head on the supporting arm could with advantage be adjusted to be functional at a lower height.

Eleven patients with psoriasis of the palms and/or soles were selected for therapy and the benefit of UV-A or UV-A+B compared with conventional tar/dithranol therapy. No patient with generalized pustular psoriasis was included but three patients had localized pustular lesions. Patients with similar lesions on both hands or on both feet were selected to give a useful index of comparison, and in-patients were studied in order to ensure no topical therapy was applied to the test area. Treatment was carried out at a distance of 40 cm, and the initial exposure time was 1 min increasing to a maximum of 10 min/day. Only two patients who were both receiving UV-A+B showed intolerance of this schedule with slight erythema.

## RESULTS

Table 1 indicates the results of comparing UV-A alone with topical tar preparations. In two patients UV-A was superior to tar, in three patients no subjective or objective difference was apparent and in one tar therapy produced a result superior to UV-A therapy.

TABLE 1. Results of UV-A and treatment to one site with no oral or topical psoralen administration. UV-A treated site is compared with tar-treated site

| Patient no. | Site treated | No. of treatments | Duration (min) | Total dose of UV-A (J/cm$^2$) | Results |
|---|---|---|---|---|---|
| 1 | Palms | 7 | 28 | 25 | UV-A ≡ Tar |
| | Backs of hands | 7 | 28 | 25 | UV-A ≡ Tar |
| 2 | Palms | 6 | 21 | 19 | Tar > UV-A |
| | Soles | 6 | 21 | 19 | UV-A ≡ Tar |
| 3 | Soles | 4 | 10 | 9 | UV-A > Tar |
| 4 | Soles | 10 | 55 | 50 | UV-A > Tar |

TABLE 2. Comparison of UV-A alone versus UV-A+B to paired sites. No topical therapy given

| Patient no. | Site treated | No. of treatments | Duration (min) | Total dose of UV-A (J/cm$^2$) | Total dose of UV-A+B(J/cm$^2$) | Results |
|---|---|---|---|---|---|---|
| 5 | Palms | 4 | 8 | 7·2 | 10·3 | UV-A > UV-A+B |
|  | Soles | 4 | 8 | 7·2 | 10·3 | UV-A > UV-A+B |
| 6 | Palms | 10 | 55 | 50 | 71 | UV-A ≡ UV-A+B |
| 7 | Backs of hands | 4 | 8 | 7·2 | 10·3 | UV-A > UV-A+B |
| 8 | Backs of hands | 8 | 46 | 41 | 60 | UV-A+B > UV-A |

Table 2 compares the use of UV-A alone with combined UV-A and B to paired sites. Of the five patients treated in this way, three showed a better response in the short term to UV-A alone. Only one patient had UV-A+B compared with conventional tar therapy, and in this patient the tar-treated area was subjectively and objectively superior to UV-A+B. Three patients who had previously shown little or no response to topical tar therapy were treated with UV-A+B to both sides and two of the three showed some benefit.

## DISCUSSION

Of the eleven patients treated, nine showed some benefit from the use of topical UV-A or UV-A+B with no additional topical therapy or systemic photosensitizer. Both non-responders were treated with combined UV-A+B. This pilot study therefore suggests that in patients with psoriasis of the palms and soles, who are reluctant to accept tar or dithranol therapy, UV-A alone is a possible method of therapy. The side-effects, both immediate and long-term, of psoralén ingestion are avoided and a clean acceptable mode of therapy can be offered. It must, however, be stated that the results of comparing UV-A or UV-A+B with currently available conventional topical therapy do not show a significant benefit from the use of UV-A alone. Clearly longer term studies are needed to determine whether or not this point holds good for patients treated for a longer period of time by a machine such as the Psorilux. At the present time it is not clear whether the long-term side-effects of PUVA are in part due to psoralens alone or are all the results of the combined action of psoralen+UV-A. It is possible that UV-A alone could in the long-term be less damaging than UV-A+ingested or topically applied psoralen.

It is recommended that minor structural alterations be made to the Psorilux to increase the safety of the operator. These include the provision of controls on a cable (5–7 ft long) distant from the actual lamp housing, the provision of better screens for the lamp and the simplification of the catch holding the filter in position.

In summary, although the Psorilux 3050A is a convenient portable apparatus which may find a place in the management of limited areas of psoriasis, the output is such that all nursing and medical personnel must be instructed in the importance of using goggles and covering exposed skin in the direct line of the lamp.

## ACKNOWLEDGMENTS

We are grateful to Dr Malcolm Davison of the Department of Clinical Physics for measuring the energy output of the Psorilux machine. Siemens Medical Group kindly made available the Psorilux used in this trial.

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# A survey of the ultraviolet radiation emissions of photochemotherapy units

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## SUMMARY

Measurements have been made of the spectral emission and irradiance from whole body ultraviolet irradiation units used in the treatment of psoriasis by photochemotherapy (PUVA). A total of ten units were studied, located at nine different treatment centres in England.

The results showed that within the limits of experimental accuracy there was no significant difference in the profile of all spectra measured, although the measured value of irradiance compared with each centre's own UV-A meter showed wide discrepancies. There is poor documentation provided by manufacturers of UV-A meters on the meaning of the scale reading in $mWcm^{-2}$, and it would seem expedient that some agreed calibration procedure for these instruments is forthcoming.

The treatment of psoriasis by photochemotherapy (PUVA) has expanded rapidly. This form of treatment involves the oral or topical administration of the photoactive drug, 8-methoxypsoralen, followed by exposure to high intensity, long-wave, ultraviolet radiation (UV-A).

A recent review (Diffey, 1978) of published papers on the photochemotherapy of psoriasis has been undertaken with particular emphasis on the specification of ultraviolet radiation dosimetry. This review showed large differences in the total UV dose needed to clear psoriatic lesions. It was concluded that these large variations can be attributed partly to an uncertain approach to the measurement of ultraviolet radiation. The problems of estimating UV irradiance using a detector whose wavelength response may not be effectively matched to the action spectrum for the regression of lesions in psoriasis have been discussed by Challoner & Diffey (1977).

The purpose of the present study was to measure the spectral emission and irradiance from treatment units at a number of centres in England in order to evaluate the consistency of radiation dosimetry. A summary of the units studied is given in Table 1. It should be emphasized that we visited only nine centres which probably represents something like 20% of the treatment centres in the U.K. at the present time and although we feel that this is a representative sample, it is possible that any conclusions drawn here may not be substantiated in a more exhaustive survey.

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TABLE 1. Details of the PUVA units studied

| Centre | Make of PUVA unit | Lamp manufacturer | Lamp designation |
|---|---|---|---|
| 1 | Rank Stanley Cox | Thorn Atlas | 75/85W UV |
| 2 | Homemade* | Thorn Atlas | 20W and 40W UV |
| 3 | SAS Chemicals Ltd | Philips | TL85W/09 |
| 4 | SAS Chemicals Ltd | Philips | TL85W/09 |
| 5 | Rank Stanley Cox | Thorn Atlas | 75/85W UV |
| 6 | Dermatron | General Electric | F72D6 |
| 7 | Rank Stanley Cox | Thorn Atlas | 75/85W UV |
| 7 | Waldmann | Sylvania | FR90T12 |
| 8 | Derma Controls | General Electric | F72T12/BL/HO |
| 9 | Waldmann | Sylvania | FR90T12 |

* Based on design of treatment cubicle 2 described by Lakshmipathi *et al.* (1977).

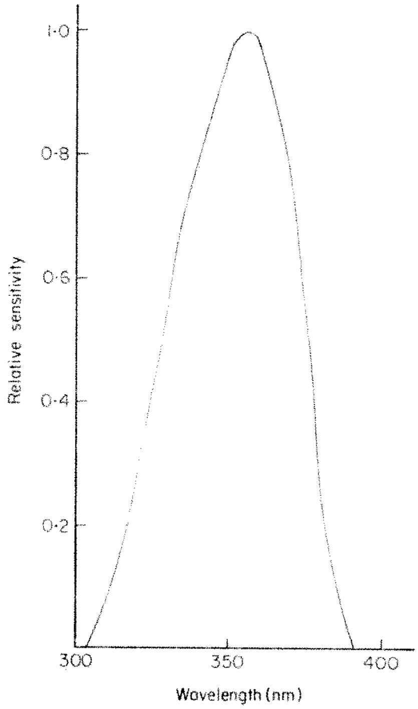

FIGURE 1. The wavelength response of an IL442A phototherapy radiometer.

## MATERIALS AND METHODS

The relative spectral emission from the lamps was determined using the scanning spectrophotometer with computer compatible output described by Diffey & Challoner (1978). Measurement of irradiance in a plane approximating that of a patient's skin was achieved as follows:

(a) The UV-A irradiance was measured using an International Light IL442A phototherapy radiometer (serial No. 791). This detector will measure the irradiance from the lamps weighted by the wavelength response shown in Fig. 1. (For a more rigorous discussion of this point see the Appendix.)

(b) The UV-A meter at each centre was also used to determine irradiance.

Steps (a) and (b) were carried out by placing each detector in turn in a specially constructed jig at approximately the position of a patient's skin. The detector height was 1 m above the podium on which the patient stands. Since the variation of irradiance is small with moderate vertical and lateral displacement of the detectors this experimental procedure is regarded as acceptable for measuring irradiance.

## RESULTS

### *Spectral emission from the lamps*

It was found that within the limits of experimental repeatability there was no significant difference in the profile of all spectra measured. Consequently for the makes of lamp specified in Table 1 the spectral emission given by Fig. 2 may be applied. This curve was derived by averaging the spectra from all units. The ordinate represents spectral irradiance in $mWcm^{-2}nm^{-1}$ when the UV-A irradiance equals 1·0 $mWcm^{-2}$. To determine the spectral irradiance for the PUVA unit at a particular centre, the ordinate scale on Fig. 2 is multiplied by the value in column 3 of Table 2 for that centre number. The average percentage radiated energy in different regions of the emission spectrum is given in Table 3.

It may be noted that there are PUVA units in clinical use which incorporate lamps with a spectral emission significantly different from that of Fig. 2, for example, the Philips actinic blue TL/05 fluorescent lamp (Lakshmipathi *et al.*, 1977) and also a medium-pressure mercury lamp used in conjunction with a Woods glass filter (Diffey & Keir, 1977). This latter lamp results in essentially monochromatic radiation at 365 nm.

### *Irradiance measurements*

The IL442A (# 791) was calibrated by the Quantum Metrology Division at the National Physical Laboratory after the field measurements had been completed. A filtered medium-pressure mercury discharge lamp was used as the calibration source. This source produces radiation in the 350–380 nm

TABLE 2. Results of irradiance measurements

| | | | UV-A meters at treatment centres | | |
|---|---|---|---|---|---|
| Centre | Hours of use | UV-A irradiance measured by IL442A (# 791) ($mWcm^{-2}$) | International Light IL442A ($mWcm^{-2}$) | Blak-Ray J-221 ($mWcm^{-2}$) | Waldmann PUVA meter ($mWcm^{-2}$) |
| 1 | 1234 | 1·76 | | 2·75 | |
| 2 | 273 | 0·93 | | | |
| 3 | 734 | 4·03 | 5·65 | 5·30 | |
| 4 | 7 | 5·31 | | 4·60 | |
| 5 | 200 | 1·97 | | 3·05 | |
| 6 | 870 | 4·41 | 2·77 | | |
| 7 | 226 | 2·31 | | 3·75 | |
| 7 | 164 | 4·13 | | 4·75 | 8·20 |
| 8 | 10 | 7·93 | 10·00 | | |
| 9 | 1810 | 2·76 | | | 2·92 |

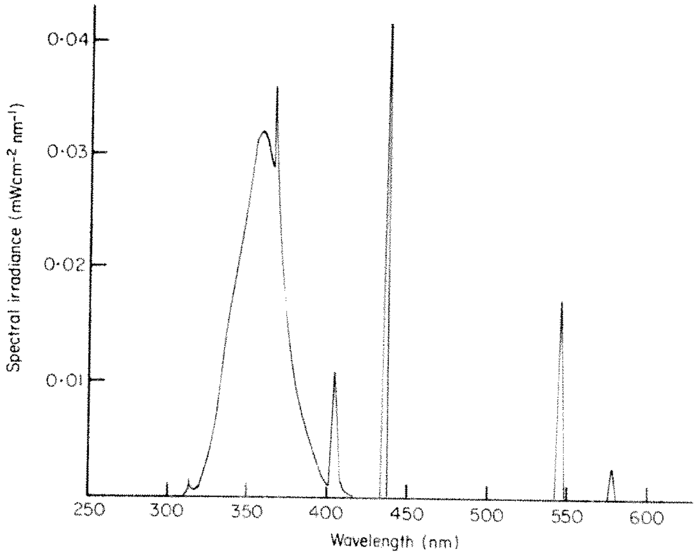

FIGURE 2. The spectral irradiance of a PUVA lamp when the UV-A irradiance is 1·0 mWcm$^{-2}$.

TABLE 3. Average radiated energy (±1 s.d.) in different regions of the emission spectrum

| Wavelength range (nm) | % Radiated energy |
|---|---|
| <315 (UV-B) | 0·43±0·32 |
| 315–400 (UV-A) | 79·80±2·80 |
| 400–600 (visible) | 12·20±1·60 |
| >600 (infrared) | 7·50±1·70 |

range, with over 97% of the radiation coming from the group of mercury emission lines between 365·0 and 366·3 nm. The calibration indicated that the IL442A (# 791) was reading high and so values of irradiance measured during the field study with this instrument were subsequently corrected. The corrected irradiance, together with the irradiance measured by the UV-A meter at each centre, are presented in Table 2.

## DISCUSSION

Examination of Table 2 indicates a wide variation in the accuracy of the UV-A meters encountered. The extremes are a meter reading 63% of the UV-A irradiance as determined by the IL442A (# 791) to a meter reading a factor of two high; a difference of over 300% in relative sensitivity.

The implication of this variability in detector response is that a patient attending Centre 1, for example, and receiving a UV dose of 5 Jcm$^{-2}$ on the basis of that centre's UV-A meter would actually receive 3·2 Jcm$^{-2}$. On the other hand a patient attending Centre 4 and again expected to receive 5 Jcm$^{-2}$ would in fact received 5·8 Jcm$^{-2}$. Although each centre would soon be able to assess the clinical efficacy of their own PUVA unit and would probably use their UV-A meter simply to monitor changes in lamp output, it is clear that comparison of UV dosimetry from the literature on PUVA treatment might be subject to some uncertainty.

The principal confusion with the UV-A meters that we looked at was that we were unable to find any documentation by the manufacturers in the operating manuals on how the meters were calibrated. It is not apparent whether the scale reading in $\mathrm{mWcm^{-2}}$ refers to an effective irradiance at 365 nm, to the total irradiance within given wavelength limits or to the weighted irradiance where the emission spectrum of the lamp is weighted by the wavelength response of the detector, with the most sensitive wavelength being attributed a weight of unity. This uncertainty leads us to suggest that it would seem expedient that some agreed calibration procedure for these instruments is forthcoming.

## ACKNOWLEDGMENTS

We are grateful to the Psoriasis Association for providing funds to allow this survey to be undertaken. Our thanks are also due to Auriema Ltd for the loan of the IL442A phototherapy radiometer.

Finally we should like to thank the staff at all hospitals we visited for making available their PUVA units and for their interest in the work.

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## APPENDIX

The UV-A irradiance at a particular point exposed to the radiation from a PUVA unit is simply equal to the integral of the spectral irradiance at that point over the UV-A range of wavelengths, i.e. 315–400 nm. This may be expressed mathematically as

$$\text{UV-A irradiance} = \int_{315}^{400} \mathrm{I}(\lambda)\mathrm{d}\lambda \ \mathrm{mWcm^{-2}} \qquad (1)$$

where $\mathrm{I}(\lambda)$ is the spectral irradiance in $\mathrm{mWcm^{-2}nm^{-1}}$ at a wavelength $\lambda$nm.

If any of the UV-A meters referred to in the paper are placed at the point of interest, the meter reading (assuming a perfect cosine response), which has been termed UV-A irradiance in the paper, will be given by

$$\text{`UV-A irradiance'} = \int \mathrm{I}(\lambda)\mathrm{S}(\lambda)\mathrm{d}\lambda \ \mathrm{mWcm^{-2}} \qquad (2)$$

where the integration is over all wavelengths for which the meter has a non-zero response. $\mathrm{S}(\lambda)$ is the spectral response of the meter at wavelength $\lambda$. Because of poor documentation by all the manufacturers referred to here on the actual interpretation of the scale reading, we assume $\mathrm{S}(\lambda)$ to have a value of unity at the most sensitive wavelength, e.g. 357 nm for the IL442A (see Fig. 1). This means that unless the radiation source emits monochromatic radiation at 357 nm, the UV-A irradiance measured by the IL442A will always be less than the true UV-A irradiance. A similar reasoning applies also to all other wavelength dependent radiometers.

If the irradiance from a PUVA unit with a spectral profile given in Fig. 2 is measured with an IL442A radiometer, the ratio of the meter reading ('UV-A irradiance') to the true UV-A irradiance will be 0·80. The irradiance measured at the same point from the same unit with a UV-A meter having a different spectral response from the IL442A will clearly result in a different 'UV-A irradiance'.

Ideally we would wish to use a detector with a spectral response which matches the action spectrum for the regression of lesions in psoriasis. However, since to our knowledge this action spectrum still remains uncertain, perhaps the next best thing would be to use a detector with a flat spectral response from 315 to 400 nm and a zero response at all other wavelengths. This detector would measure true UV-A irradiance irrespective of the spectral power distribution of the source. Unfortunately such a detector does not exist for various physical reasons (although it could be approximated by means of a thermopile and appropriate cut-off filters).

Nevertheless presently-used UV-A meters can still enable us to compute the true UV-A irradiance or even some biological irradiance (assuming a PUVA action spectrum is postulated) if we have access to the relative spectral profile of the radiation source and the spectral response of the UV-A meter. Should a careful comparison be required of the therapeutic results from centres using radiation sources of different spectral power distribution or different UV-A meters, then we believe it to be important that these comments be borne in mind.

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**Pharmacology and Treatment**

# Razoxane (ICRF 159) in the treatment of psoriasis

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## SUMMARY

This paper describes our experience with the use of razoxane (ICRF 159), a new anti-mitotic drug, in the treatment of thirty-six patients with severe psoriasis. The drug has proven highly effective both in cutaneous and in arthropathic disease. The drug is well tolerated by patients and appears to be free of hepatotoxicity. However, it causes depression of the neutrophil leukocyte count, an effect which is entirely dose-dependent; the hazard to patients can be minimized by careful supervision of therapy.

Razoxane, (ICRF 159) (±) 1,2-bis (3,5-dioxopiperazin-1-yl) propane, is a relatively new anti-tumour drug which is a derivative of ethylenediamine tetra-acetic acid (EDTA), though there is no evidence that its therapeutic effect depends on chelation. It is unique in arresting cell cycle progression only during the late pre-mitotic ($G_2$) or the early mitotic (M) phase of the cell cycle (Sharpe, Field & Hellmann, 1970).

In certain experimental animal tumours razoxane has profound anti-metastatic activity (Hellmann & Burrage, 1969), which may result from a specific effect on the microvasculature of the primary tumour. In solid malignant tumours the overall microvascular architecture is disorganized; individual vessels are tortuous and show irregular dilation; wide gaps are seen between the endothelial cells. Treatment with razoxane has been shown to result in the 'normalization' of this type of microvasculature in primary tumours (Le Serve & Hellmann, 1972) and it is proposed that this change reduces the escape of tumour cells into the circulation. Folkman (1972) has shown that tumour growth depends upon rapid neovascularization, which is itself dependent upon the release by tumour cells of an endothelial mitogen. In psoriasis, rapid epidermal cell proliferation is associated with a microvasculature resembling that seen in solid malignant tumours (Braverman, Cohen & O'Keefe, 1972) and Folkman (1972) has suggested that an analogous reciprocal relationship might exist between the proliferative cells and the microvasculature in such lesions. If this were the case, treatments having an inhibitory effect on the abnormal microvasculature could be of therapeutic benefit.

The possibility of a therapeutic role for razoxane in psoriasis was suggested by the following:

(1) It inhibits a wide range of tumours when used as a single agent (Bakowski, 1976).
(2) Its microvascular effect might be of special relevance in psoriasis.
(3) It is given by mouth and is generally well tolerated.

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(4) It appears to be free from the unpredictable complications which are characteristic of the cancer chemotherapeutic agents in current use for severe psoriasis.

In 1976 we published our initial results with this drug in the treatment of psoriasis (Atherton, Wells & Hellmann, 1976). These were sufficiently promising to encourage us to use the drug in further patients when indicated, and several of these patients have now received treatment for 2 years or longer. The wide interest that has been shown in this drug in relation to psoriasis has prompted the present interim report.

## PATIENTS AND METHODS

A total of thirty-five patients with severe psoriasis were treated with razoxane. Thirty-two were treated principally for incapacitating cutaneous disease (see Table 1) which had failed to respond to topical therapy and particularly to dithranol. Three of these patients also had fairly severe arthropathy while minor degrees were common. A further three patients were treated primarily for severe arthropathy which had not responded to non-steroidal anti-inflammatory drugs.

TABLE 1. Type of psoriasis in thirty-five patients treated with razoxane

| | | |
|---|---|---|
| Principally cutaneous disease | | 32 |
| Nummular | 11 | |
| Erythrodermic | 15 | |
| Acropustulosis (Hallopeau) | 2 | |
| Generalized pustular (von Zumbusch) | 1 | |
| Palmoplantar pustulosis | 3 | |
| Principally arthropathy | | 3 |
| Total | | 35 |

Sixteen patients had already received other anti-mitotic drugs, and some had also had photochemotherapy. All of these sixteen had received methotrexate for varying periods, but without control of the disease in nine cases, and with the development of adverse effects necessitating cessation of treatment in the other seven. In one case, this had consisted of intractable nausea and vomiting; in another, profound and recurrent neutropenia. The remaining five had developed hepatic abnormalities. In two of these cases serum levels of hepatic enzymes were persistently elevated with fatty change only on liver biopsy; and in the other three, liver biopsy revealed hepatic fibrosis, with or without elevated serum levels of hepatic enzymes.

Razoxane was not given to patients of either sex who planned to have children within the next 2 years. All patients undertook to use adequate contraception. The patients were all informed of the known adverse effects of the drug and it was explained that its long-term safety remains unconfirmed.

Razoxane was administered as 125 mg tablets. Figure 1 illustrates the various dosage schedules used. Initially, low doses were usually given; recently we have preferred schedule 6 during this phase. After 4 weeks, the dosage could gradually be increased by stages at no less than monthly intervals until a satisfactory therapeutic effect was achieved, or dose-limiting side-effects appeared. During maintenance therapy, razoxane was given for either 2 or 3 consecutive days each week, or in a few patients, each fortnight. The daily dose was always either 125 mg or 250 mg three times daily. Hospital admission for treatment was not necessary in the majority of cases.

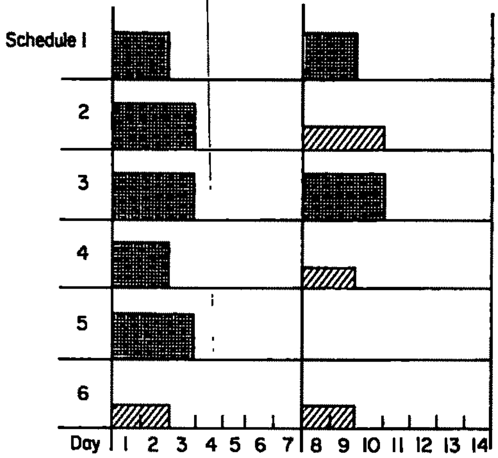


FIGURE 1. Razoxane dosage schedules. Stippled blocks—250 mg three times daily; hatched blocks—125 mg three times daily.

Estimations of haemoglobin concentration, differential white cell count and platelet count were undertaken in all patients before treatment. Biochemical screening included determination of serum creatinine, urea, uric acid, aspartate aminotransferase, alkaline phosphatase, bilirubin, albumin and total protein. Urinary creatinine clearance was estimated in all patients. Liver biopsy was performed in all patients who had previously received methotrexate, though neither abnormalities of liver function nor biopsy appearance were considered a contra-indication to treatment with razoxane.

All patients had a urinary creatinine clearance in excess of 75 ml/min.

Patients were seen after the first course of treatment, then at fortnightly intervals for approximately 6 weeks, and subsequently at intervals varying between 4 and 8 weeks. Joint disease was assessed clinically and three parameters were recorded:

(i) the duration of early morning stiffness (EMS);
(ii) the 'Ritchie Index', an established scoring system for joint tenderness (Ritchie *et al.*, 1968);
(iii) the number of 'active' joints, as manifest by synovial swelling with or without an effusion.

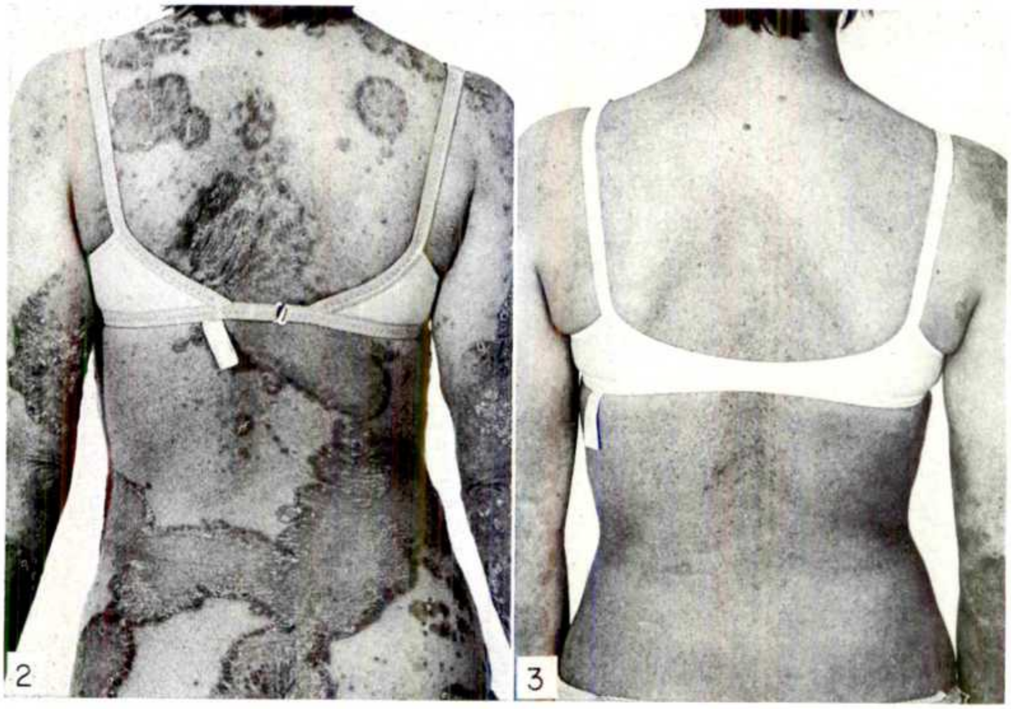
All patients were required to have a further haemoglobin estimation, differential white cell count and platelet count at least every 4 weeks. Administration of razoxane was temporarily stopped and re-started in reduced dosage in any patient in whom the neutrophil count fell below $1 \cdot 2 \times 10^9$/l. Regular biochemical screening was performed in all patients.

Four patients were subjected to bone marrow aspirations both before and after approximately 6 weeks of treatment. One of these patients was subjected to a further marrow aspiration 11 months after the cessation of treatment. Two patients have had liver biopsies after 2 years of treatment.

## RESULTS

### *Cutaneous psoriasis*

No patient failed to respond clinically to treatment with razoxane. Regression of psoriasis to mild or minimal residual disease occurred in thirty-one out of the thirty-two (97%) patients receiving treatment primarily for cutaneous disease (Figs 2–5). Difficulty in obtaining adequate control of cutaneous



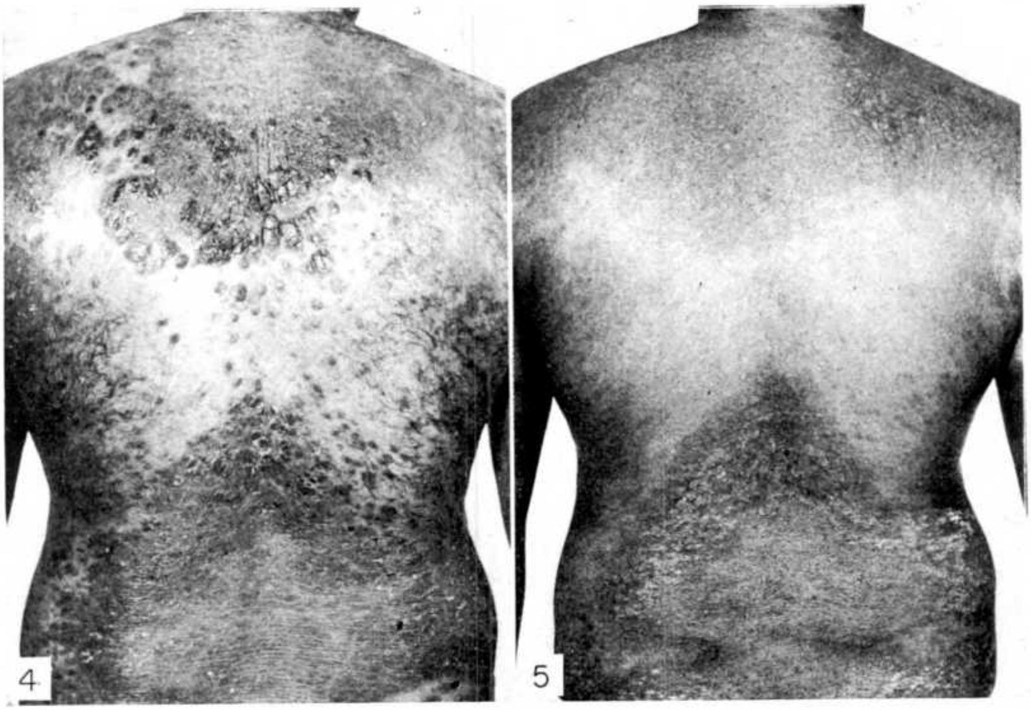
FIGURES 2 AND 3. Back of a 36-year-old woman (2) before treatment; (3) after 4 months' treatment.

psoriasis was encountered in only one patient, a 63-year-old woman with unstable erythrodermic psoriasis affecting about 70% of her total skin area. Clinical improvement was accompanied by development of alopecia, nausea, diarrhoea and colicky abdominal pain, and subsequently neutropenia, forcing a reduction of dosage. The alopecia, nausea and diarrhoea improved but her psoriasis recurred as the dosage was reduced. Treatment was therefore stopped and has not been reinstituted.

To minimize bone marrow toxicity, it was decided to give the drug intermittently rather than continuously. We are currently using six dosage schedules in our patients (see Fig. 1). Schedule 1 appears to be the optimal regime for most men of average weight who are under the age of 60. Some men, usually those under 40 years of age, may require 3-day rather than 2-day courses and schedules 2 or 3 are often preferable in these cases. Most women under the age of 60 are best treated with schedule 4, though those under the age of 40 may require higher dosage in the form of schedule 1. Schedule 5 is useful for patients in whom arthropathy is the major problem. Schedule 6 is often the most suitable for older, frailer patients of both sexes, and is now preferred for initial treatment in all cases. Although the optimal schedule can usually be forecast for patients on the basis of age, sex, weight and the severity of disease, individual patients' requirements vary considerably. The aim of treatment is the suppression of disease to a mild level which is acceptable to the patient. The high dosage required to produce complete clearance exposes the patient to an unnecessarily high risk of toxicity.

### *Psoriatic arthropathy*

Several of the thirty-two patients being treated principally for cutaneous psoriasis had concurrent minor joint symptoms, most commonly in the finger and toe joints. Complete suppression of these



FIGURES 4 AND 5. Back of a 37-year-old man (4) before treatment; (5) after 3 months' treatment.

symptoms was the rule within the first few weeks after starting treatment. Six patients had more severe degrees of arthritis, and in three of these joint disease constituted the principal indication for treatment with razoxane. Data relating to the treatment of this small sub-group are shown in Table 2 and Figs 6–9.

## ADVERSE EFFECTS

### *Bone marrow toxicity*

Some degree of bone marrow depression is an invariable accompaniment of treatment with razoxane, except in patients on low dosage schedules.

(a) *Neutropenia.* Neutropenia due to bone marrow depression is the commonest haematological side-effect encountered and is frequently dose-limiting. The neutrophil count usually falls sharply at the initiation of treatment, reaching a new level at 1·5–2·5 × $10^9$/l. The mean neutrophil count of the thirty-five patients at the start of treatment was 6·0 × $10^9$/l, falling to 2·1 × $10^9$/l after 6 months of treatment. During treatment occasional patients record neutrophil counts below 1·5 × $10^9$/l; this is usually a transient effect and does not *per se* necessitate any alteration in dosage. Neutrophil counts below 1200 × $10^9$/l have been recorded in fourteen patients, below 1·0 × $10^9$/l in eight patients and below 0·4 × $10^9$/l in three patients. The nadir of the neutrophil count occurs between 3 and 10 days following the last dose. Following cessation of treatment the neutropenia has always recovered, the neutrophil count rising above 1·2 × $10^9$/l within 12 to 13 days after the last dose. Two of the three patients whose counts fell below 0·4 × $10^9$/l developed pyrexia and were treated in hospital with intravenous antibiotics during the period preceding recovery.

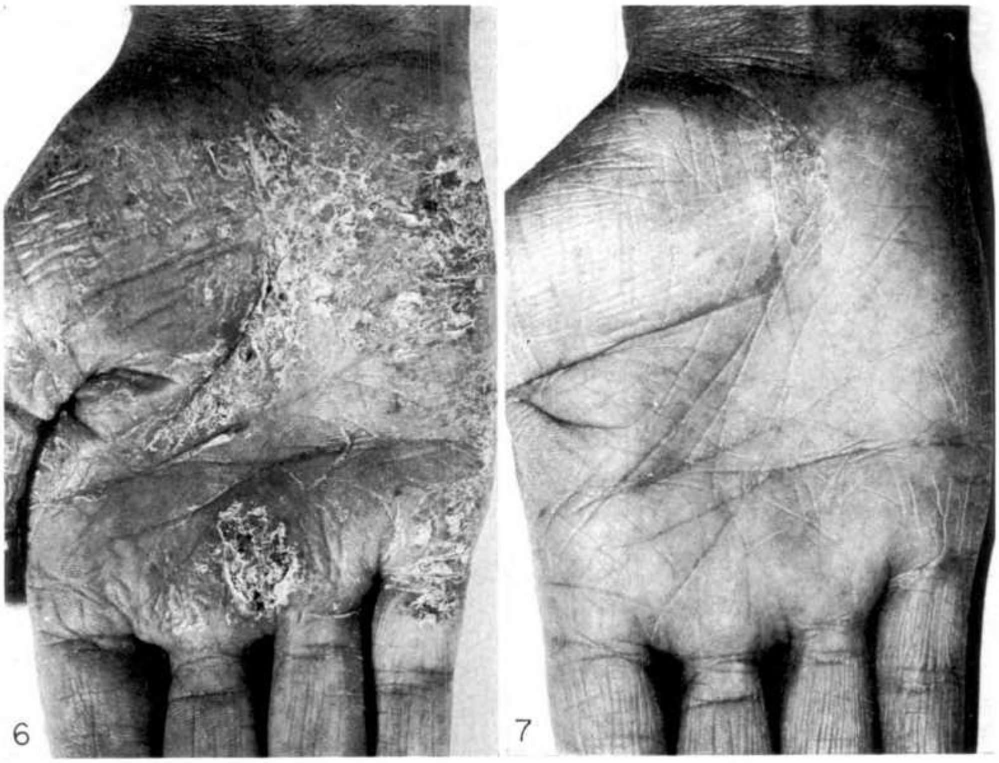
TABLE 2. Clinical details of six patients with more severe degrees of psoriatic arthropathy

| Patient | Age | Sex | Duration of psoriasis (years) | Duration of arthritis (years) | Joints involved | Type of psoriasis | Previous treatment | Duration of treatment (months) | Razoxane administration schedule |
|---|---|---|---|---|---|---|---|---|---|
| 1. D.P. | 42 | M | 17 | 18 | Wrists, M.C.P.s, P.I.P.s, knees, M.T.P.s | Nummular | N.S.A.I.D., azathioprine, methotrexate | 24 | 2 |
| 2. M.B. | 26 | M | 5 | 4 | M.C.P.s, P.I.P.s, knees, hips, ankles, M.T.P.s, cervical spondylitis | Guttate | N.S.A.I.D. | 24 | 5 |
| 3. A.H. | 30 | M | 13 | 13 | M.C.P.s, wrists, shoulders, knees, hips, M.T.P.s, sacroiliitis, thoraco-lumbar spondylitis | Erythrodermic | N.S.A.I.D., prednisolone, methotrexate | 18 | 2 |
| 4. P.R. | 32 | M | 21 | 6 | Wrists, M.C.P.s, P.I.P.s, knees, hips, ankles, M.T.P.s | Erythrodermic | N.S.A.I.D., gold salts, methotrexate | 14 | 1 |
| 5. V.W. | 58 | F | 30 | 26 | Wrists, M.C.P.s, knees, ankles, cervical spondylitis, sacroiliitis | Erythrodermic | N.S.A.I.D., gold salts, prednisolone | 6 | 4 |
| 6. M.P. | 54 | F | 20 | 15 | M.C.P.s, wrists, shoulders, knees, ankles | Erythrodermic | N.S.A.I.D., gold salts, cortisone | 6 | 4 |

M.C.P.—metacarpo-phalangeal joints; P.I.P.—proximal inter-phalangeal joints; M.T.P.—metatarso-phalangeal joints: N.S.A.I.D.—non-steroidal anti-inflammatory drugs.

A few giant neutrophils with hypersegmented nuclei are commonly seen in the peripheral blood of patients during treatment. Typically these cells show an approximately 30% increase in diameter above normal, having between five and eight nuclear lobes, and coarse clumped nuclear chromatin with very fine interlobar connections. The significance of this finding will be discussed later.

(*b*) *Anaemia*. A moderate reduction in haemoglobin concentration is common. The mean value in



FIGURES 6 AND 7. Palm of a 40-year-old woman (6) before treatment; (7) after 3 months' treatment.

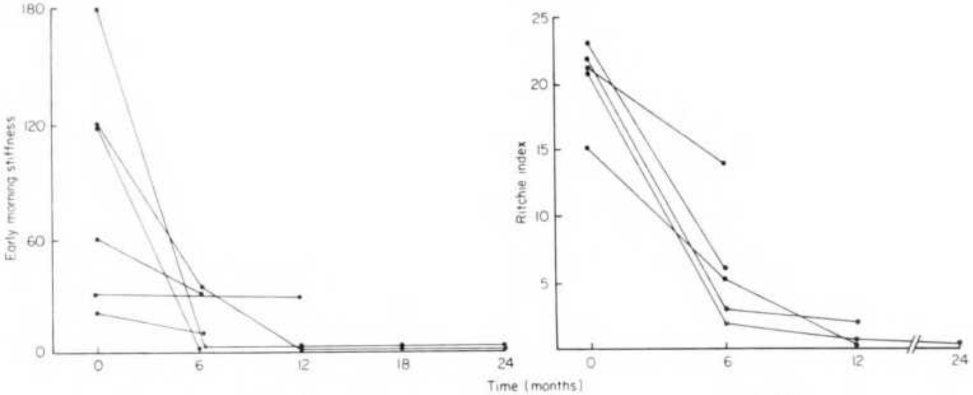
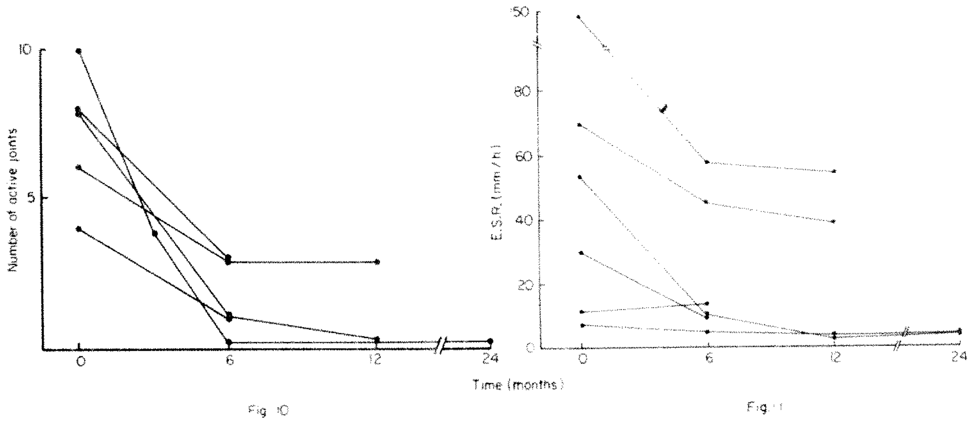


Fig 8

Fig 9

FIGURES 8 AND 9. Effects of treatment on (8) early morning stiffness; (9) the Ritchie index in six patients with psoriatic arthropathy.



FIGURES 10 AND 11. Effects of treatment on (10) the number of active joints; (11) the E.S.R. in six patients with psoriatic arthropathy.

our patients at the initiation of treatment was 14·7 g/dl; falling after 6 months of treatment to 12·8 g/dl (a mean fall of 1·9 g/dl). No red cell abnormalities have been seen in peripheral blood films.

Although slight falls in the platelet count are often recorded in patients on treatment, no count has been recorded below $120 \times 10^9$/l, the lower limit of normal in our laboratory.

(*c*) *Histological bone marrow changes.* The appearance in the peripheral blood of giant neutrophils with hypersegmented nuclei prompted bone marrow examinations in a small number of patients, both before and after a period of treatment. Data were obtained in four informed volunteers. All had normal serum folate and vitamin $B_{12}$ levels. All marrow examinations were essentially normal at the start of treatment and all showed similar changes after periods of treatment varying between 2 and 40 weeks. The overall cellularity of the bone marrow was markedly reduced; granulopoiesis was depressed; giant metamyelocytes, myelocytes and promyelocytes were present, showing the same coarse clumping of nuclear chromatin observed in the peripheral blood neutrophils; erythropoiesis was normoblastic; occasional giant late normoblasts were present showing karyorrhexis and prominent basophilic stippling of the cytoplasm.

In one of these patients we had the opportunity to reexamine the bone marrow 11 months after cessation of a 3-month course of treatment; the appearances were then completely normal, suggesting that the changes described above are normally entirely reversible.

### *Alopecia*

A degree of diffuse alopecia was almost invariable in patients receiving the higher dosage regimes for periods exceeding 3 months. This effect was usually not reported by the patients unless severe; this being the case in only four patients (11%); in none was the alopecia total. Improvement in the degree of hair loss always followed reduction in dosage, and complete recovery occurred in one patient whose treatment was for other reasons ceased. In many cases, gradual improvement was observed without modification of dosage.

### *Nausea*

Seven of the thirty-five patients (20%) experienced nausea at some time during treatment. This occurred most commonly in patients being treated with 3-day courses, on the third day, and occasionally during the subsequent 1 or 2 days. In all except one patient the nausea was transient and of mild degree. In this patient the nausea recurred intermittently over a period of several months, being accompanied eventually by vomiting which necessitated withdrawal of treatment for its relief.

### *Diarrhoea*

Three patients (9%) experienced diarrhoea at some time during treatment. In all these cases the drug was probably responsible since, on most occasions, the diarrhoea occurred during the third day of 3-day treatment courses and during the subsequent 1 or 2 days, as with the nausea. In no case was the diarrhoea profuse. In two of the three patients affected the diarrhoea was transient but in the third it persisted until the cessation of treatment.

### *Lethargy*

Lethargy was reported by seven patients (21%) at some time. The development of this symptom was usually associated with the higher dosage regimes and it also tended to occur on the third day of 3-day treatment courses and during the subsequent 1 or 2 days. This symptom was usually accompanied by the more severe degrees of bone marrow depression, and can be regarded as a fairly reliable indication that this has occurred. Changing to 2-day treatment courses led in every case to its relief.

### *Headaches*

Diffuse headache was reported by two patients (6%), being described as a 'dull ache' in both cases. As with the other symptoms described, headache occurred in patients on 3-day high dose treatment courses, on the third and subsequent one to two days. In both cases it was associated with lethargy and responded to a change to 2-day courses.

### *Blood-stained nasal discharge*

This was an unexpected symptom reported by six patients (17%). These patients described an uncomfortable sensation in the nose, associated with a thin, slightly blood-tinged discharge. This usually occurred in the winter, and symptoms were generally confined to the morning; in no case was it persistent. The two patients with the most marked symptoms were examined by an ENT surgeon, but no significant abnormality was apparent. We assume that a slight degree of atrophy of the nasal mucous membrane was responsible.

### *Menstrual irregularity*

Two patients associated the development of menstrual abnormalities with treatment. One woman, aged 38, developed amenorrhoea which has persisted at the time of writing for 6 months. The other patient, aged 40, has experienced light menses of short duration but occurring every 2 weeks, which have now occurred for 4 successive months.

The frequent association of unwanted symptoms with the third day of treatment in patients on 3-day courses led us to substitute 2-day courses where feasible. This reduction in the number of treatment days rarely resulted in decreased therapeutic efficacy, but did lead to a reduction in both subjective and objective (haematological) toxicity.

No patient has shown any biochemical evidence of hepatic toxicity. Those patients who had raised serum levels of liver-associated enzymes at the start of therapy, as a result of methotrexate-induced liver damage, have shown gradually falling or static levels since starting razoxane. None of these patients has shown rising levels of such enzymes during razoxane treatment. Two patients have had normal liver biopsies after 2 years of treatment. One of these had a normal liver biopsy at the start of treatment, having previously been on methotrexate for just over 1 year; the other had never received methotrexate, and had not had a pre-treatment biopsy. We plan to perform further liver biopsies in patients who have received treatment for over 2 years.

## DISCUSSION

In terms of both therapeutic efficacy and toxicity razoxane has proved a promising agent in the treatment of severe psoriasis. We believe that it is at least as effective as methotrexate. In only one patient with cutaneous psoriasis (3%) has a worthwhile therapeutic effect not been achieved, because of dosage limitations imposed by the early onset of symptomatic toxicity. It was particularly satisfying that a good effect was achieved in eight of the nine patients referred to us because of their previous poor response to methotrexate. Patients with arthropathy showed similar marked benefit, with little remaining evidence of active joint disease after 3 months of treatment.

As with other antimitotic drugs, toxicity may be a prominent feature. The principal problem is depression of bone marrow function which affects particularly production of neutrophil leukocytes. This effect appears to be entirely dose-dependent and even severe degrees of neutropenia have always resolved rapidly on cessation of treatment. Although depression of bone marrow function is more conspicuous than with methotrexate, hepatotoxicity has not so far been seen, nor has it been reported in the large numbers of patients treated for malignant disease. The further liver biopsies we plan will provide valuable data in this respect.

The drug is very well tolerated, better, in our experience, than methotrexate. Symptomatic toxicity, when it occurs, is usually mild and in only two patients (6%) has it necessitated cessation of treatment.

The optimal dosage schedules with razoxane have yet to be established. Initially we gave the drug in 3-day courses at weekly intervals because there was considerable experience of the use of this schedule in the field of oncology. We subsequently decided to evaluate 2-day courses because of the success of the so-called 'Weinstein-Frost' regime for the treatment of psoriasis with methotrexate (Weinstein & Frost, 1971). This regime, in which methotrexate is given in three doses over 36 h, is now widely considered to be the optimal schedule for this drug, though the validity of the rationale on which its use is based, in terms of epidermal cell proliferation kinetics, is now seriously questioned (Wright, 1976). Our experience shows that 2-day courses are effective in the majority of patients, and are associated with reduced toxicity, particularly with respect to the bone marrow. On each treatment day, the razoxane is given in sub-divided doses because it has been shown (Creaven, Allen & Alford, 1975) that this results in better gastro-intestinal absorption than single daily doses. Patients being treated principally for arthropathy appear to obtain optimal relief of symptoms with 3-day courses; the proliferation kinetics of psoriatic synovium may differ from those of psoriatic epidermis.

The finding of giant neutrophil leukocyte and erythrocyte precursors in the bone marrow of treated patients, and the related finding of giant neutrophils in the peripheral blood, are consistent with the specific inhibitory activity of this drug at the $G_2/M$ phase of the cell cycle. DNA synthesis continues normally but nuclear division is inhibited (Hallowes, West & Hellmann, 1974). From a theoretical point of view, inhibition of the $G_2/M$ phase of the cell cycle would not be expected to result in mutagenicity, and this is supported by the absence of any mutagenic effect in the 'Ames test' (McCann *et al.*, 1975; Garner, unpublished data). It is believed that mutagenicity in this test correlates well with carcinogenicity (Ames, Lee & Durston, 1973) and razoxane would therefore be predicted to be non-carcinogenic in contrast to many other anti-cancer drugs, including methotrexate. Razoxane was tested for its ability to induce primary lung tumours in strain A mice (Stoner *et al.*, 1973). There was no evidence of carcinogenicity in this test.

A small study was recently performed by the National Cancer Institute in the U.S.A., in which an increased incidence of uterine adenocarcinomata and systemic lymphomata appeared to follow alternate daily intraperitoneal administration of very large doses of razoxane (48 and 96 mg/kg) to rats and mice respectively (DHEW Publication (NIH), 78–1328, 1978)*. The relevance of these findings is uncertain because the dosage, the route of administration and the administration schedules in these studies were entirely different from those employed in our own patients. Nevertheless, the possibility is raised that this drug may have some carcinogenic potential, and this has to be weighed against the likely benefits of therapy in individual patients.

## ACKNOWLEDGMENTS

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* Because of these findings, the manufacturers of the drug are unable to recommend the use of razoxane in psoriasis. The drug is available for use in certain types of malignant disease, and dermatologists or rheumatologists who prescribe it for patients with psoriasis do so entirely on their own responsibility.

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# Photochemotherapy and topical nitrogen mustard in the treatment of mycosis fungoides

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## SUMMARY

Eight patients with mycosis fungoides limited to the skin have been treated with either topical nitrogen mustard alone or in combination with photochemotherapy. This regime does not prevent contact sensitization to nitrogen mustard but will clear sanctuary sites of disease which may develop when photochemotherapy is used alone.

Mycosis fungoides is an infiltration of the skin with thymus derived (T) lymphocytes and may eventually prove fatal through dissemination to lymph nodes and other organs. Eight patients with early stage disease (I and II) have been treated with topical applications of nitrogen mustard alone or in combination with photochemotherapy.

Nitrogen mustard therapy for mycosis fungoides is very effective. It is widely accepted in the United States and long-term remissions have been reported (van Scott & Kalmanson, 1973; Price *et al.*, 1977). However, contact dermatitis is frequent (40%) and this has detracted from its use in this country (Samman, 1976). Photochemotherapy of mycosis fungoides has recently been described (Gilchrest *et al.*, 1976; Hodge *et al.*, 1977) and involves taking 8-methoxypsoralen orally before exposure to long-wave ultraviolet light (PUVA). Complete clearing of the disease undoubtedly occurs but it appears that treatment is only temporarily suppressant although the optimum therapeutic schedule has yet to be worked out. PUVA therapy is lymphocytotoxic. Various workers (Lischka *et al.*, 1977; Edelson *et al.*, 1977; Cormane *et al.*, 1977; Ortonne *et al.*, 1977) have demonstrated that therapeutic doses of 8-methoxypsoralen plus UV-A alter lymphocyte function *in vivo* and *in vitro*. It was considered that combination of PUVA with nitrogen mustard therapy might so depress lymphocyte function as to prevent sensitization of the skin to the latter. The purpose of this communication is to report that PUVA therapy does not prevent sensitization, to draw attention to the development of sanctuary sites of disease if photochemotherapy is used alone and to record that combination of topical nitrogen mustard with photochemotherapy will clear these sanctuary sites of disease.

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## PATIENTS AND METHODS

Eight patients with extensive cutaneous mycosis fungoides were treated. In all cases the diagnosis was confirmed by biopsy and the skin lesions conformed to either Stage I or II disease (van Scott & Kalmanson, 1973). Thus in Stage I disease, erythematous patches and minimally infiltrated plaques were present and in Stage II moderately infiltrated plaques were found in addition. General medical examination was unrevealing and in particular there was no lymphadenopathy or hepatosplenomegaly. The full blood count, urea, electrolytes, liver function tests and chest X-rays were normal. Lymphocyte transformation to phytohaemagglutinin (PHA) was performed since normal responsiveness has been shown to correlate with early stage disease (du Vivier *et al.*, 1978a). All the patients lymphocytes responded normally to PHA, indicating that their disease was limited to the skin.

Of the eight patients, three have had topical nitrogen mustard therapy alone, four commenced photochemotherapy and nitrogen mustard simultaneously and one had photochemotherapy for 2½ years before commencing nitrogen mustard therapy. The nitrogen mustard was applied daily. The photochemotherapy was given three times weekly. Some patients applied the mustard before photochemotherapy and others after. No specific instructions as to timing were given.

The method used for nitrogen mustard therapy is as follows. The patient is taught to draw up into a syringe 10 ml of tap water which is introduced into a 10 mg vial of nitrogen mustard. The vial is shaken and the 10 ml withdrawn and put into a cup. A further 40 ml of water is added so that the final concentration is 20 mg%. This is immediately applied to the entire cutaneous surface by the patient, the whole process taking about 5 min to perform. Applications are made daily until 6 months after complete remission and then approximately twice weekly for 3 years (Vonderheid *et al.*, 1977).

The photochemotherapy patients received between 0·3–0·5 mg 8-methoxypsoralen per kg body weight 2 h before exposure to UV-A. A Rank Stanley Cox machine was used. The initial dose was determined by the skin type of the patient and ranged from 1·5–4·5 J/cm$^2$ for each treatment.

## RESULTS

Of the three patients receiving nitrogen mustard therapy alone, two sensitized to the mustard and developed a contact dermatitis within 1 month. One of these had previously received 200 $\mu$g of nitrogen mustard intravenously once a week for 5 weeks prior to topical therapy in an attempt to induce tolerance as advocated by van Scott & Kalmanson (1973). Both these patients were, however, successfully treated with topical desensitization as described by Constantine, Fuks & Farber (1975) and were able to tolerate applications of 20 mg% of nitrogen mustard once again within 2–3 months of starting treatment.

Of the four patients who received topical nitrogen mustard and photochemotherapy simultaneously, all patients were cleared of visible skin disease. Two patients, however, developed contact dermatitis to the nitrogen mustard. One became sensitized after 5 weeks of daily applications of mustard. He had received 32 J/cm$^2$. It has not been possible to desensitize him topically. He is now receiving photochemotherapy alone and is free of visible disease except in his groins and between some of his toe webs. The other patient sensitized after 3 months of daily mustard therapy. He had received 80 J/cm$^2$ at this time. He has been successfully desensitized topically.

The patient who had been receiving photochemotherapy for 2½ years initially had an excellent response. However, it was then observed that she had developed lesions of mycosis fungoides on her upper eyelids, between her fingers, under her breasts and in her natal cleft and groin. She was therefore started on topical nitrogen mustard therapy whilst continuing the photochemotherapy but developed a contact dermatitis after 5 weeks of therapy. She had received 260 J/cm$^2$ of UV-A at this time. However, she was successfully desensitized and is now completely free of disease in the sanctuary sites and elsewhere. Photochemotherapy has been stopped and she uses nitrogen mustard therapy alone.

## DISCUSSION

The results show that combination therapy does not diminish the incidence of sensitization to nitrogen mustard in that three out of the five patients became sensitized. This is disappointing particularly in view of the report by Volden, Molin & Thomsen (1978) that contact dermatitis diminished after PUVA therapy. However, as we commented at the time (du Vivier & Vollum 1978), this was probably due to anergy resulting from disease progression, since it was a number of years after the original sensitization to mustard that photochemotherapy and subsequently mustard therapy were given to the patients.

A very important observation has been made, however, and must cause some concern about the use of photochemotherapy alone. Plaques of mycosis fungoides were observed in sites shielded from irradiation where no disease had been visible prior to therapy and indeed in sites where it is unusual for mycosis fungoides to occur. These areas we have termed sanctuary sites. Similar observations have been made by other workers (Samman, personal communication; Beare, personal communication). The explanation of lesions in these sites is not clear. It is possible that the malignant lymphocytes have migrated from treated areas to those naturally shielded from irradiation. Alternatively it may be that free multiplication of malignant T cells occurs where there is no suppressive or lymphocytotoxic effect from PUVA. Photochemotherapy is at an experimental stage for the treatment of mycosis fungoides but these findings are disturbing enough to question whether the present treatment regimes are the correct approach to therapy.

Fortunately lesions in these sites can be easily cleared using nitrogen mustard therapy, since the entire skin is treated including areas which are shielded from irradiation. It is also a simpler method of therapy than photochemotherapy and four of the combination therapy patients have stopped receiving photochemotherapy. Contact dermatitis is an undoubted disadvantage but it was possible to desensitize some of the patients. Cutaneous malignancies have been recorded as developing after several years of exposure to nitrogen mustard (du Vivier *et al.*, 1978b). This is a serious disadvantage of therapy. The lesions, however, were diagnosed early and were easily treated. A similar risk is associated with photochemotherapy (Stern *et al.*, 1979). On the other hand mycosis fungoides is an unpleasant and malignant condition and alternative safer therapies are lacking.

This study indicates that nitrogen mustard therapy remains an effective treatment for mycosis fungoides and that concomitant PUVA therapy does not prevent the occurrence of contact sensitization. Photochemotherapy clears the treated skin of visible disease but disease is subsequently to be found in areas shielded from irradiation. The addition of topical nitrogen mustard therapy will clear the santuary sites of disease.

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***Case Reports***

# Erythromelanosis follicularis faciei et colli

## CASE REPORTS

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### SUMMARY

Erythromelanosis follicularis of the face and neck has seldom been described in the literature. It is a condition of unknown aetiology. The clinical features are reddish-brown pigmentation, telangiectatic vessels, and pale follicular papules localized in the peri-auricular area. The disease is asymptomatic and has only been described in men. This report records two patients with erythromelanosis follicularis of the face and neck, a boy and a young adult female.

According to Ebling & Rook (1972) this syndrome of unknown aetiology was originally described in Japan by Kitamura *et al.* (1960). All affected individuals were men, young and middle-aged, who had suffered from pigmentary disturbances since puberty or early adolescence.

This report records two patients with erythromelanosis follicularis of the face and neck. One of the patients (Case 2) is a woman.

### CASE REPORTS

*Case* 1

A 12-year-old boy with no family history of skin diseases. From early childhood he had a pronounced reddish-brown pigmentation involving the peri-auricular area, both cheeks and the chin (Fig. 1). There were sparse changes on the forehead. The border of the lesions was well demarcated. There was no loss of eyebrows. In the affected areas there was a strong tendency to sweating. No scar formation or scaling was observed. On the upper extremities there was pronounced keratosis pilaris. The facial lesions remained unchanged although during winter, especially in cold weather, they exacerbated a little. Other exogenous factors were not found.

The changes consisted partly of pale pinhead size follicular papules, and partly of a reddish-brown pigmentation which was most prominent between the papules. Diascopy revealed a fading of the reddish component and persistence of the brown pigmentation.

The symptoms were mostly of a cosmetic nature, but the sweating tendency also caused complaints in that the patient's spectacles had to be replaced every year because of rust formation.

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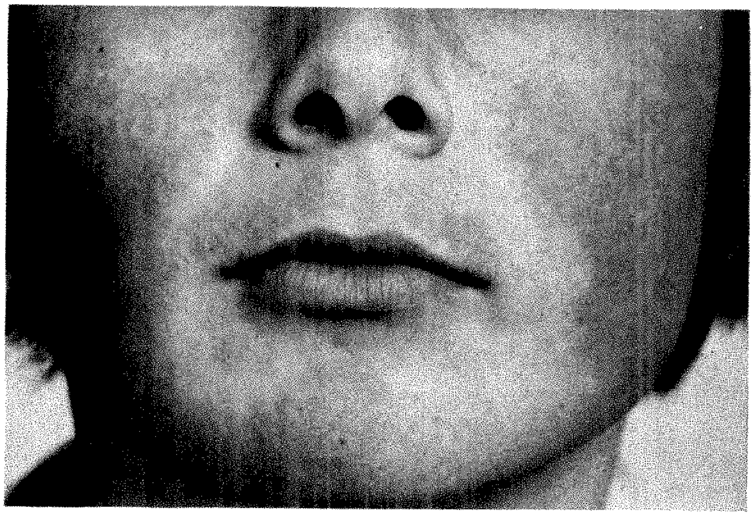

FIGURE 1. Well demarcated reddish-brown pigmentation in Case 1.

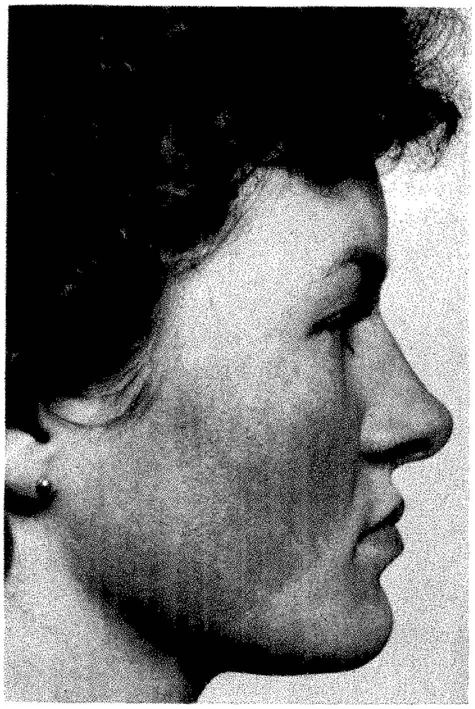

FIGURE 2. Case 2.

Histological examination revealed slight hyperkeratosis and acanthosis with no parakeratosis. There was pronounced pigmentation of the stratum basale, especially seen with Fontana staining.

The follicles were enlarged and showed infiltration of round cells in their surroundings. In the dermis chronic inflammatory cell infiltration and oedema were present.

The patient was treated for two months with topical retinoic acid. The follicular keratosis and the red discoloration decreased, but due to subjective complaints treatment was discontinued.

### *Case 2*

A 21-year-old woman with no family history of skin diseases. Since puberty she had had a reddish-brown discoloration on both cheeks extending to the neck. There were no changes on the forehead or chin. The border of the lesions was well demarcated (Fig. 2). No scarring, scaling or inflammatory changes were observed. The facial lesions have remained unchanged and do not exacerbate in cold or warm weather. No exogenous factors were found.

The changes consisted of dense follicular micro-papules. Pigmentation was most prominent between the papules. Diascopy revealed a fading of the reddish component and persistence of the brown pigmentation. Keratosis pilaris was present on the upper arms.

The patient had no subjective complaints, especially no increased sweating tendency.

Histological findings revealed slight hyperkeratosis without parakeratosis. There was pronounced pigmentation in the stratum basale, seen especially with Fontana staining. The follicles were enlarged and showed moderate fibrosis. A large number of telangiectatic vessels was found in corium, surrounded by heavy infiltration of lymphocytes and plasma-cells.

The patient was treated with topical retinoic acid. The lesions faded in colour and the follicular keratosis decreased. However, the changes did not disappear completely.

## DISCUSSION

Kitamura *et al.* (1960) described the disease in six Japanese men between the ages of 17 and 22 years, and Mishima & Rudner (1966) in four Caucasian men. It has been postulated that this condition may not be uncommon in Caucasians. It has not been possible, however, to find other cases of erythromelanosis follicularis of the face and neck described in the literature.

The two patients reported here showed typical clinical changes in the form of peri-auricular reddish-brown pigmentation with telangiectatic vessels and pale, follicular papules. The histological findings do not differ from those previously described. However, up till now this condition has only been described in men.

Differential diagnoses of erythromelanosis follicularis of the face and neck are ulerythema ophryogenes, keratosis pilaris of the face and erythrose peribuccale pigmentaire of Brocq. The two diagnoses first mentioned differ from erythromelanosis follicularis in blanching completely on diascopy and by the lack of melanin pigmentation in the stratum basale. Furthermore, ulerythema ophryogenes presents scaling and scarring. Erythrose peribuccale pigmentaire differs from erythromelanosis follicularis in lacking follicular papules and in being scaly.

The two cases reported vary from those earlier described in the following: Case 1 had an early onset and a pronounced sweating tendency. Case 2 is a female.

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# The development of pseudo-Kaposi's sarcoma after placement of a vascular access graft

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## SUMMARY

Pseudo-Kaposi's sarcoma developed on the skin overlying a vascular access graft in a haemodialysis patient. After spontaneous thrombosis within the graft, the cutaneous lesions promptly resolved. Light and electron microscopic study of the lesions failed to distinguish them from those of true Kaposi's sarcoma.

Pseudo-Kaposi's sarcoma and Kaposi's sarcoma are angiomatous diseases that share certain clinical and histological features. Both may present as haemorrhagic nodules on an extremity and both are characterized by marked vascular proliferation (Bluefarb & Adams, 1967; Stewart, 1967; Stewart & Lauret, 1970; Earheart *et ol.*, 1974). They differ in their biological behaviour. Kaposi's sarcoma is a low grade, slowly progressive, multifocal neoplasm which occasionally may metastasize (Sanderson, 1972) while pseudo-Kaposi's sarcoma is a reactive, self-limited process associated with arteriovenous malformations (Earhart *et al.*, 1974).

## CASE REPORT

A 67-year-old Filipino male was seen at the Long Beach Veterans Administration Medical Center in 1977 for red nodules on his arm. Past medical problems included polycythaemia rubra vera diagnosed in 1967 and treated with multiple phlebotomies and melphelan until 1972. In 1973, the patient underwent a transurethral resection of the prostate with bilateral orchiectomies for a well-differentiated adenocarcinoma. Adult onset diabetes was managed without medication. Hypertension, congestive heart failure, and atrial fibrillation were controlled with lasix and digoxin.

Chronic renal insufficiency of undetermined etiology was discovered in 1965. Because of progressively diminishing renal function, a left brachial artery expanded polytetrafluorethylene arteriovenous shunt was placed in April 1977.

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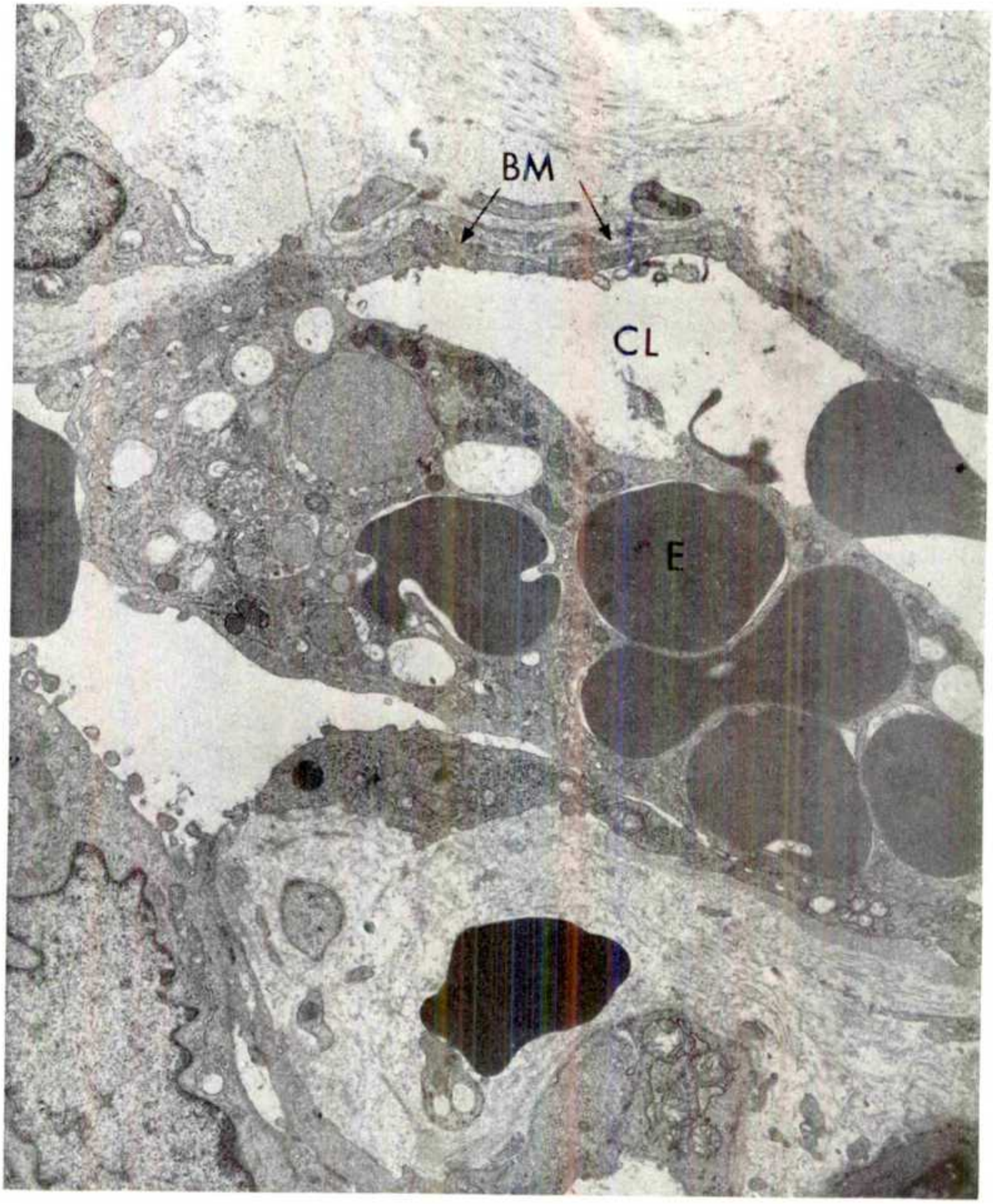

FIGURE 1. Erythrophagocytic endothelial cell containing many pinocytotic vesicles. Erythrocyte within membrane bound vacuole (E). Capillary lumen (CL). Basement membrane zone (BM) (× 10,000).

In September 1977, several firm violaceous papules 2–7 mm in diameter developed on the volar aspect of the left forearm. Within 1 month, fourteen papules were present.

The majority of the papules were located around the distal 10 cm of the graft loop. No papule was more than 2 cm from a limb of the graft.

Light microscopic examination of haematoxylin and eosin (H & E) stained sections from a punch biopsy of these lesions showed epidermal hyperkeratosis and atrophy. Beneath a thin zone of normal dermis and extending deeply was a densely cellular fibrovascular tumour. It was composed of randomly oriented bundles of spindle cells that formed numerous vascular slits. Their nuclei showed moderate variation in size and staining characteristics. Occasional mitotic figures were noted, some having atypical configurations. Erythrocytes had extravasated into the tumour's stroma and at the periphery of the lesion there was a mild inflammatory infiltrate composed of lymphocytes, histiocytes and plasma cells.

For ultrastructural examination, material was fixed in 2% phosphate buffered gluteraldehyde, post-fixed in 1% osmium tetroxide for 30 min, dehydrated, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate. Two cell types predominated. The most common was a phagocytic endothelial cell. These cells were surrounded by pericytes and a continuous basement membrane in well developed vessels. The endothelial cells were characterized by rod-shaped microtubular bundles, multivesicular bodies, and by numerous micropinocytotic vesicles along the plasma membrane and within the cytoplasm. The majority of the vessels were immature with hypertrophied endothelial cells often distorting the lumen. Around these vessels, basement membrane was deposited irregularly and pericytes were absent. Widening and absence of tight junctions were also noted. The other common cell type was a spindle shaped or stellate fibrohistiocytic cell. The fibrohistiocytic cell contained no microtubular bundles and fewer micropinocytotic vesicles than the endothelial cells. Both cell types showed erythrophagocytosis with erythrocytes lying within membrane-bound vacuoles (Fig. 1). Digestion of erythrocytes yielded vacuoles filled with granular electron-dense material interpreted as ferritin.

Prior to clinical resolution, a sodium pertechnetate technitium 99 m (15 mCu) blood pool imaging and total body rectilinear scan was performed. The shunt area of the left forearm showed increased blood pooling and scan activity consistent with the clinical lesions. The remainder of the scan was unremarkable.

In mid-November the fistula spontaneously thrombosed. Marked flattening of the papules occurred within 2 weeks. During the next 2 months the lesions continued to involute until only 2 hyperpigmented macules remained.

A second polytetrafluoroethylene graft was placed in January 1978 using the right brachial artery. No lesions have developed in the overlying skin during the 1 year follow-up interval.

## DISCUSSION

The association of an underlying congenital arteriovenous malformation with a cutaneous dysplastic angiopathy which may resemble Kaposi's sarcoma, both clinically and histologically, is well known. Stewart & Lauret (1970) and Earhart *et al.* (1974) used the term pseudo-Kaposi's sarcoma to apply to such lesions. Baxt, Mori & Hoffman (1975) reported the development of Kaposi's sarcoma-like lesions 3 years after the appearance of an arteriovenous fistula of the hand. Following surgical closure of the fistula, these lesions resolved.

Less frequently reported, however, are cases of pseudo-Kaposi's sarcoma iatrogenically induced. Chandon *et al.* (1975) reported the development of pseudo-Kaposi lesions in a haemodialysis patient after placement of an arteriovenous vascular access graft. As in our patient, lesions developed in close proximity to the graft within a few months after placement.

The graft implanted in our patient was a single large-calibre expanded polytetrafluoroethylene graft (Gore-Tex, W.L. Gore, Newark, Delaware). Polytetrafluoroethylene (PTFE) is a porous, synthetic, non-biogradable material that has been gaining popularity as a vascular prosthesis. Unlike other materials, PTFE allows fibroblast ingrowth into its wall and the formation of an endothelial neo-intimia (Elliott *et al.*, 1977). Because vessels cannot penetrate the walls of graft, we assume the lesions are not due to vascular outgrowths from the graft.

The development of Kaposi's sarcoma in patients in renal failure has been associated with the use of immunosuppressive drugs (Stribling, Weitzer & Smith, 1978). Although there is evidence to show that renal failure itself may cause immunosuppression (Holdsworth *et al.*, 1978), we believe that the development of our patient's cutaneous disease shortly after placement and in close proximity to a vascular axis graft is no coincidence. Certainly, its prompt resolution immediately after thrombosis of the graft supports the notion that this lesion is a reactive dysplastic angiopathy of the pseudo-Kaposi type.

The presence of nuclear atypia and vascular slits have been thought to distinguish Kaposi's sarcoma from other angiomatous neoplasms (Earhart *et al.*, 1974; Lever & Schaumburg-Lever, 1975); our patient demonstrated both these findings.

Ultrastructurally, major features of Kaposi's sarcoma are a proliferation of immature vascular channels (Hashimoto & Lever, 1964) and endothelial and fibroblastic cell populations (Mottaz & Zelickson, 1966). Erythrophagocytosis by endothelial, pericytic and fibroblastic cells has been emphasized as a particularly characteristic finding (Waldo *et al.*, 1977). Our patient's lesions exhibited all these features.

In an informal local survey of over 100 patients on haemodialysis with similar vascular access grafts, no further lesions were seen nor were nephrologists in our community aware of any similar cases.

In conclusion, although our patient's lesions were histologically identical to Kaposi's sarcoma, they evolved in a clinical setting which would support the contention that they represented a benign, dysplastic angiomatosis—pseudo-Kaposi's sarcoma.

## ACKNOWLEDGMENTS

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# Immunohistochemical findings in a patient with penicillamine pemphigus

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## SUMMARY

This report describes the immunofluorescence (IF) findings in sequential skin biopsies and serum obtained during a follow-up study of a patient who developed pemphigus lesions after D-penicillamine treatment of arthritis. The distribution in the skin of IgG, IgM and C3 varied with the date, type and site of the biopsy sample. Thus IF patterns showed features of (a) pemphigus vulgaris, (b) pemphigus foliaceus and (c) pemphigus erythematosus. Indirect IF examination indicated the presence of circulating intercellular epithelial antibody of both the IgG and IgM class.

Pemphigus-like eruptions have been described in patients treated with penicillamine for over 10 years (Dégos *et al.*, 1969). Various reported cases of penicillamine pemphigus had intercellular epithelial deposition of IgG in the skin, but recently two patients have been described with concomitant IgG deposits along the basement membrane zone (Sherak, Kolarz & Holubar, 1977; Kennedy, Hodge & Sanderson, 1978). The immunohistological features in these two cases appear very similar to those found in pemphigus erythematosus (Chorzelski, Jablonska & Holubar, 1968; Bean & Lynch, 1970). The studies reported so far have usually presented immunofluorescence results of single biopsies only.

We describe the immunohistochemical findings in sequential skin biopsies obtained during a follow-up study of 9 months in a patient with penicillamine pemphigus.

## CASE REPORT

### *Clinical course*

A 57-year-old man had a history of severe rheumatoid arthritis. He was treated with D-penicillamine 250 mg three times daily from December 1975. In April 1978 he developed an itchy eruption on the back, chest, abdomen and upper arms.

Flaccid bullae appeared repeatedly giving rise to numerous discrete scaly and crusted erythematous

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plaques and superficial erosions, 1–3 cm in diameter. The lesions were evenly distributed on the back, chest, abdomen, upper arms, and later on the scalp. His face and oral mucosa were not affected at any time. The diagnosis of penicillamine induced pemphigus was made, and in May 1978 D-penicillamine was discontinued. The lesions responded well to Jamarsan therapy (Schiff, 1961). However, new lesions have been appearing occasionally, also on the lower extremities.

### *Histopathology*

A biopsy taken from a blister showed bulla formation with acantholytic cells at a high level in the epidermis, consistent with the diagnosis pemphigus foliaceus.

### *Laboratory findings*

Routine tests revealed no abnormalities, except for ESR 134 mm/h. Serum albumin 29 g/l (normal 34–47 g/l). Levels of IgG, IgA, C4 and C1-INH were normal; IgM 2·56 g/l (normal 0·7–2·0 g/l), C3 1·756 g/l (normal 1·265 ± 0·162 g/l).

Serological tests for rheumatoid arthritis: latex agglutination 1:10,000 and Rose test 1:1024. VDRL, cardiolipin and RPCF tests were negative. Anti-nuclear antibody: homogeneous type, IgM class, titre 1:100 to 1:1000, anti-kappa 1:100, anti-lambda negative. (ANA tests were repeatedly negative before starting penicillamine therapy.) Anti-dsDNA negative. HLA typing: A2, Aw19, B5, B15, Bw4, Bw6, Cw3, Cw4, DRw1, DRw6(?).

## IMMUNOFLUORESCENCE PROCEDURES

Skin biopsies for direct immunofluorescence (IF) examination were snap frozen in liquid nitrogen and processed as reported elsewhere (Terpstra, de Jong & Klokke, 1979) using commercially obtained fluorescein isothiocyanate conjugates with the following specificities:

Goat anti-human IgG (Kallestad), P 11·2 mg/ml
AbP 2·16 mg/ml, F/P ratio 2·2 M; working dilution 1:80
Goat anti-human IgM (Kallestad), P 11·5 mg/ml
AbP 2·08 mg/ml, F/P ratio 2·4 M; working dilution 1:40
Goat anti-human IgA (Kallestad), P 16·6 mg/ml
AbP 1·55 mg/ml, F/P ratio 1·5 M; working dilution 1:40
Horse anti-human C3, B1C/B1A (Kallestad), P 10·1 mg/ml
AbP 1·24 mg/ml, F/P ratio 2·5 M; working dilution 1:16
Rabbit anti-human C1q (Behringwerke AG), P 10 ± 3 mg/ml
AbP 1·1 ± 0·1 mg/ml, F/P ratio 2·5 M; working dilution 1:30

The monospecificity of the anti-Ig conjugates was confirmed in a performance testing system using bone marrow preparations (Faulk & Hijmans, 1972). Additional positive and negative controls included skin specimens obtained from patients with contact dermatitis, pemphigus vulgaris, dermatitis herpetiformis and systemic lupus erythematosus. Serum was obtained at monthly intervals and stored at −80°C until examined by indirect IF using human skin and guinea-pig lip as antigenic substrates and the above anti-human IgG and IgM conjugates.

Examination of cryostat sections was carried out using a Leitz Orthoplan microscope equipped with a XBO 75 W lamp and a Ploemopak-2 vertical illuminator for incident-light excitation. We used position 3 of the vertical illuminator.

TABLE 1. Direct IF staining reactions* for IgG, IgM and C3 in lesional and uninvolved skin

| Skin biopsy specimen | | | Intercellular epith. struct. | | | Dermo–epidermal junction | | | Vessel wall structures | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Type | Site | IgG | IgM | C3 | IgG | IgM | C3 | IgG | IgM | C3 |
| 25/4/78 | Lesion | Upper arm | 3+ | ± | 3+ | + | + | ± | – | 2+ | 2+ |
| | Normal | Forearm | 2+ | – | ± | – | 2+ | ± | – | ± | + |
| 17/9/78 | Lesion† | Lower trunk | | | | | | | | | |
| | Normal | Forearm | 2+ | ± | + | 2+ | 3+ | + | ± | 2+ | 2+ |
| 17/10/78 | Lesion | Upper back | 3+ | – | + | – | 2+ | 2+ | – | 2+ | 2+ |
| | Normal | Upper back | 2+ | – | – | – | + | + | – | + | 2+ |
| 6/12/78 | Lesion | Lower trunk | 3+ | + | 2+ | – | 2+ | – | – | 2+ | 2+ |
| | Normal | Forearm | 3+ | 2+ | 2+ | 2+ | 3+ | 3+ | ± | 3+ | 3+ |
| 7/12/78 | Lesion | Lower trunk | 2+ | ± | 3+ | ± | 2+ | 2+ | – | 2+ | 2+ |
| | Normal | Forearm | 2+ | + | 2+ | 2+ | 3+ | 2+ | ± | 3+ | 3+ |

* Staining reactions: – negative; ± doubtful; +/2+/3+ positive.
† Biopsy unsatisfactory.

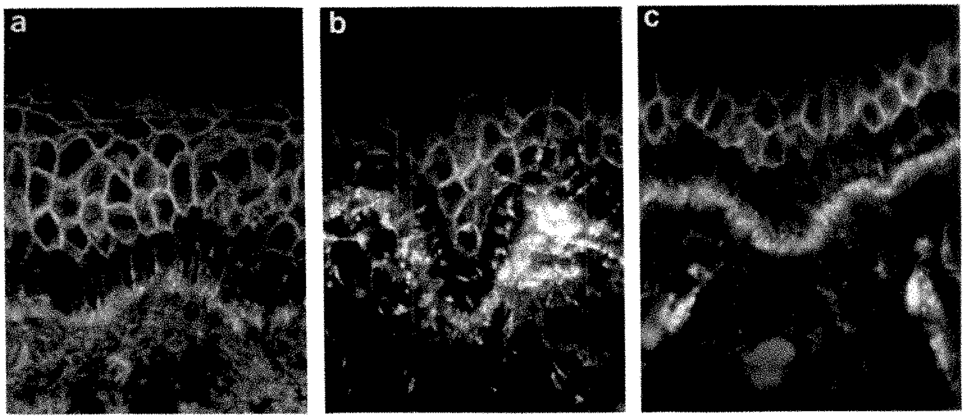

FIGURE 1. Cryostat sections of uninvolved skin from the forearm (December 1978) showing the intercellular epithelial and the junctional zone staining pattern for (a) IgG, (b) IgM and (c) C3. Note also deposition of IgM and C3 in the walls of superficial blood vessels (direct IF staining; oil immersion, ×25).

## RESULTS

### *Direct IF*

The results of staining reactions for IgG, IgM and C3 (B1C/B1A) in four lesional and five uninvolved skin biopsies, obtained during a follow-up study of 9 months, are presented in Table 1.

Intercellular (IC) deposition of IgG was found in two main patterns, one covering all layers of the epidermis (not shown) and one confined to the upper layers only (Fig. 1a). These patterns occurred both in lesional and in clinically normal skin. Intercellularly bound IgM and C3 were most prominent in the middle part of the epidermis (Fig. 1b, c).

Deposition of IgG, IgM and C3 was also noted along the dermo–epidermal junction (DEJ) in a partly granular, partly homogeneous pattern. In two out of nine biopsies the concomitant presence of these serum proteins was detectable both in the IC structures and along the DEJ (Fig. 1a–c). In two lesional skin specimens, IgG positive plasma cells were present in the upper dermis. Superficial blood vessel walls showed predominantly granular staining for IgM and C3.

Staining for IgA appeared negative in all instances, whereas staining for C1q produced a very finely granular fluorescence of weak intensity in the upper dermis. This staining pattern was however also noted in control skin specimens. For comparison, distinct granular staining was observed with IgA in the dermal papillae in dermatitis herpetiformis, and with C1q along the DEJ in systemic lupus erythematosus.

*Indirect IF*

Circulating IC antibodies of the IgG class were detectable in titres ranging from 1:40 to 1:160, using guinea-pig lip as antigenic substrate. Furthermore, the patient's serum was found to produce a granular IC staining pattern for IgM when using normal human skin as substrate (Fig. 2a), suggesting the additional presence of circulating IgM-class IC antibodies in a titre of 1:16. No such staining was observed in parallel skin sections treated with normal human serum, or with more recently obtained serum samples of the patient (Fig. 2b). We failed to demonstrate the presence of IgM-class anti-C3 immunoconglutinin.

## DISCUSSION

A man with seropositive R.A. and negative ANA tests after treatment with D-penicillamine developed skin lesions consisting of subcorneal bullae changing into crusted, scaly macules. Direct IF patterns for IgG, IgM and C3, observed in nine skin biopsies, varied with the date, type and site of the biopsy sample, revealing characteristics of pemphigus vulgaris, pemphigus foliaceus (Bystryn & Rodriquez, 1978) and of pemphigus erythematosus (Chorzelski *et al.*, 1968; Bean & Lynch, 1970).

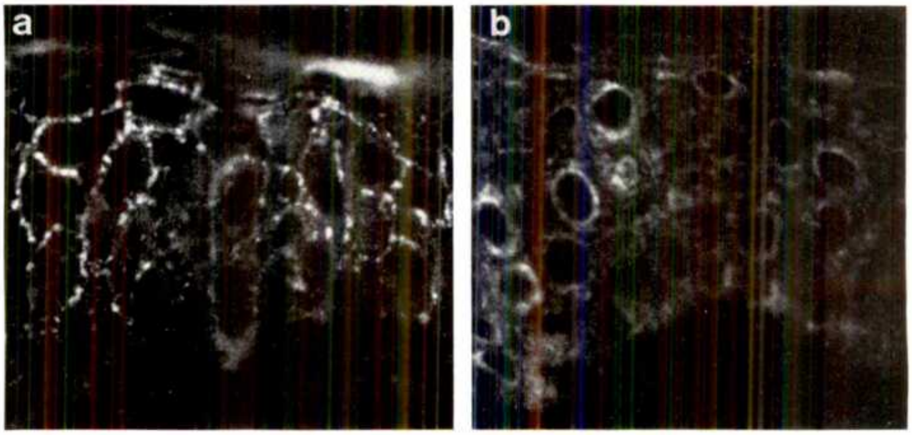

FIGURE 2. Cryostat sections of normal human skin treated with the patient's serum (1:8) and stained for the presence of IgM. (a) Granular fluorescence of intercellular epithelial structures following incubation with serum in December 1978; (b) absence of intercellular staining after treatment with serum in February 1979 (indirect IF staining; water immersion, ×45).

Interestingly, we found both *in vivo* IC deposition of IgM and transient circulating IgM class antibody reacting with IC structures in normal human skin, which has not been described before.

It was not possible to classify the skin symptoms definitely as either pemphigus erythematosus or pemphigus foliaceus. The borderline between these entities in the literature is vague (Lever, 1965). Our patient had no lesions of the face, and the scales were not typical leaf-like. There has been no tendency to generalization so far. This might support a diagnosis of pemphigus erythematosus. The IF findings were at times in agreement with this diagnosis. However, it seems hard to use this term in a patient without any lesions suggestive of L.E.

The unique aspect of our case was the observation of variable IF patterns in the skin at different times over a 9-month period. The variety of IF patterns found may not be surprising in view of the fact that penicillamine may induce pemphigus vulgaris, pemphigus erythematosus, pemphigus foliaceus (Hewitt, Benveniste & Lessana-Leibowitz, 1975; Sherak *et al.*, 1977; Kennedy *et al.*, 1978), and also S.L.E. and positive ANA tests (Camus *et al.*, 1975).

Obviously it is necessary to study a larger number of patients before and during treatment with D-penicillamine.

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# Salmonella gastroenteritis—another cause of erythema nodosum

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Although there are many causes and associations of erythema nodosum, it remains a useful sign often giving a clue to the diagnosis of a puzzling illness. It is therefore important for clinicians to be aware of all the possible causes and associations. The following report is of a patient in whom typical erythema nodosum developed during the course of severe salmonella gastroenteritis.

## CASE REPORT

This 33-year-old female civil servant was in excellent health until September 1978 when, after staying at a hotel in Newcastle, she started to have diarrhoea and intermittent abdominal pain. Other guests at the hotel were similarly affected. At worst her bowels were opened thirty times a day. The stool was like thin mushroom soup and contained no blood. After she had had diarrhoea continuously for 17 days, during which time she lost 6 kg in weight, painful red nodules appeared on both shins. They were typical of erythema nodosum and they persisted for 10 days to be replaced by areas of bruising. One week after the rash appeared the left ankle became painful, red, and swollen. This gradually settled after a week. At the time the rash appeared she had a temperature of 39°C and she was admitted to hospital where she was treated with intravenous fluids containing potassium supplements, and ampicillin 500 mg intramuscularly 6-hourly. Nothing was allowed by mouth. The only treatment before admission was loperamide hydrochloride. She had never taken oral contraceptives. One week after admission she was passing a normal formed stool twice daily. She was then discharged home taking oral ampicillin for 2 weeks and she made an uncomplicated recovery. When last seen 3 months later she was asymptomatic.

The following investigations were done during the illness: haemoglobin 13·2 g/dl, white cell count $12{\cdot}1 \times 10^9/l$, erythrocyte sedimentation rate 50 mm in 1 h; plasma potassium 2·9 mmol/l; serum alkaline phosphatase 326 iu (normal <250 iu), other liver function tests were normal; ASO titre 50 units; stool culture isolated *Salmonella typhimurium* sensitive to ampicillin, blood cultures did not isolate *Salmonella typhimurium*; sigmoidoscopy showed an apparently normal mucosa with the normal vessel pattern; rectal biopsy showed an oedematous lamina propria infiltrated by chronic inflammatory cells, polymorphs infiltrated the glandular and surface epithelium and the latter showed reactive changes with goblet cell depletion.

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The following investigations were done after recovery: haemoglobin 14·5 g/dl, white cell count $8\cdot1 \times 10^9$/l, erythrocyte sedimentation rate 2 mm in 1 h; serum alkaline phosphatase 148 iu; serum B12 474 ng/l (normal 250–1200 ng/l), serum folic acid 3·3 μg/l (normal 3–15 μg/l), red cell folate 213 μg/l (normal 160–600 μg/l), ASO titre 100 units; stool culture was negative for *Salmonella typhimurium* on three occasions; sigmoidoscopy to 15 cm was normal; rectal biopsy was normal; barium meal and follow-through, barium enema and chest X-ray were normal.

## DISCUSSION

Typical erythema nodosum of the shins with arthralgia occurred during the course of a typical, albeit severe and prolonged, attack of salmonella gastroenteritis. This association has not been described previously. Indeed, the combination of diarrhoea and erythema nodosum is said usually to signify ulcerative colitis (Spiro, 1977). It was therefore thought important to exclude inflammatory bowel disease which, furthermore, may coexist with salmonella infection (Dronfield, Fletcher & Langman, 1974). Subsequent investigations have excluded this condition as far as is possible.

The pathogenesis of erythema nodosum is not fully understood. There is probably a vasculitis provoked by a local immunological response to one of a variety of antigens. Immunofluorescent staining of a biopsy from affected skin, looking for the presence of a recognizable antigen (in this case a salmonella antigen), would advance our knowledge of the pathogenesis and should be considered in cases in which a recognizable antigen is suspected.

## ACKNOWLEDGMENT

I would like to thank Dr David Jenkins, consultant pathologist, for his help.

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# Idiopathic calcinosis cutis of the penis

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## SUMMARY

Calcification of the penis, whether in the skin, subcutaneous tissue or corpora is exceedingly rare and is usually secondary to trauma or Peyronie's disease. A case of idiopathic calcinosis cutis of the penis is presented.

## CASE REPORT

A 13-year-old boy presented with two nodules on the skin of the penis which had been present for 9 months (Fig. 1). There was no history of trauma and no relevant family history. Physical examination revealed no other physical abnormality.

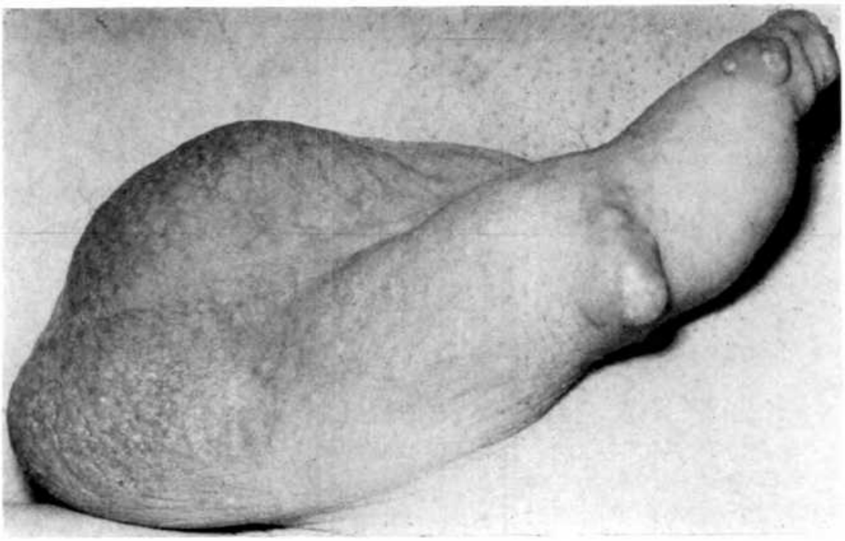

FIGURE 1. Lesions on penile skin.

Correspondence: Dr Abel.

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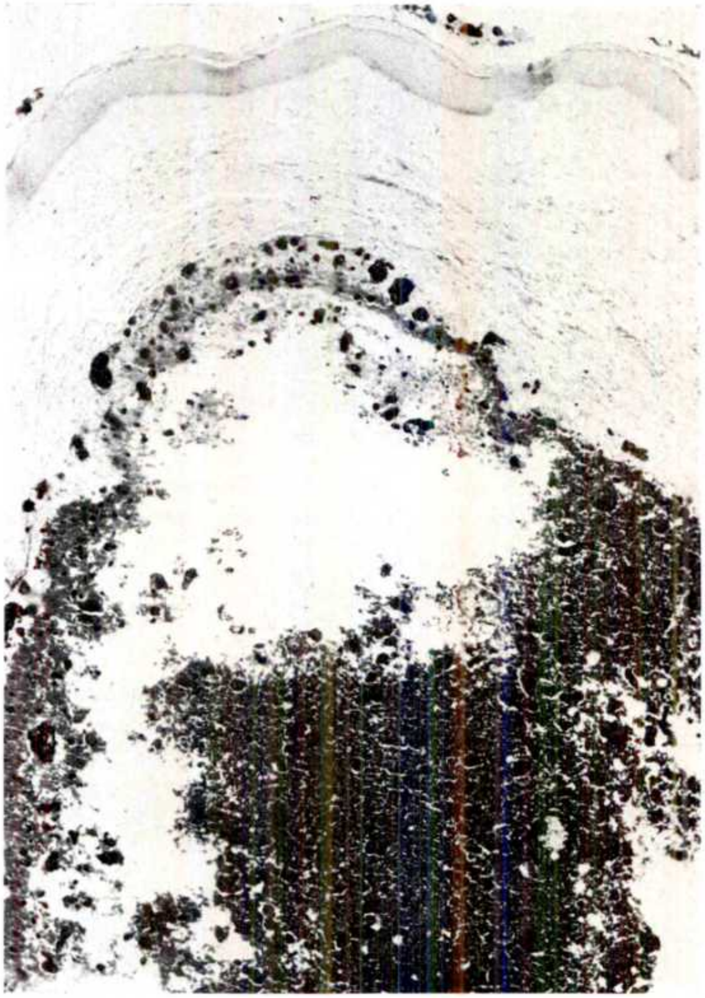

FIGURE 2. Histology of lesion showing deposits of calcification and the presence of foreign body giant cells (H & E, × 44).

Haemoglobin and E.S.R., blood urea, serum calcium, electrolytes, phosphorus, urate, acid and alkaline phosphatases were all within normal limits.

A diagnosis of retention cysts was made and the lesions were excised under general anaesthesia.

At histological examination (Fig. 2) the epidermis was normal and no cysts were present. The dermis was widely infiltrated by small deposits of calcification from 1 to 10 mm in diameter. There was a marked foreign body giant cell reaction. At infra-red spectroscopy calcium carbonate and calcium apatite were demonstrated.

Healing was satisfactory and at follow-up examination 3 months post-operatively no new calcified deposits could be detected.

## DISCUSSION

Pathological calcification may be broadly divided into two groups, metastatic and dystrophic. The former is associated with an increase of either serum calcium or phosphorus levels or both. In childhood hyperparathyroidism soft tissue calcification outside the kidney is rare (Wilkins, 1965) but if present usually involves lung, heart or arteries. Skin involvement is exceptional (Putkonen & Wangel, 1959). Dystrophic calcification is usually secondary to trauma, tissue necrosis or diffuse connective tissue disease.

Dystrophic calcification of the male genitalia is uncommon although cases have been reported involving the penile musculature (Joffe, 1961), the testes (Priebe & Garret, 1970), and the scrotum (Fisher & Dvoretsky, 1978; Shapiro, Platt & Torres-Rodriguez, 1970).

Dystrophic calcification in the penis is rare but has been described following trauma (Hecker, 1844), in association with Peyronie's disease (Joffe, 1961) and as a side-effect of cytotoxic therapy (Ihde *et al.*, 1975).

Our case of idiopathic calcinosis cutis of the penis is, we believe, unique to the literature.

## ACKNOWLEDGMENT

We would like to acknowledge the assistance of Dr A.C.A.Glen, Consultant Biochemist, for his help with the infra-red spectroscopy.

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# Multiple Pacinian neurofibroma and relationship with the finger-tip arterio-venous anastomoses

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## SUMMARY

Multiple Pacinian neurofibromas involving the three phalanges of the ring finger of the left hand in a 60-year-old woman are described. The abnormal proliferation of Vater-Pacini corpuscles in various stages of differentiation was associated with marked changes of the glomus type of arterio-venous anastomoses of the finger-tip. The close relationship between the new proliferated Vater-Pacini corpuscles, their altered baresthetic function and the pathological shunting of blood account for the painful Raynaud-like symptoms. The name 'Pacinian neurofibromatosis' is proposed.

A close topographical and functional relationship between the finger-tip arterio-venous anastomoses (AVA) and the Vater-Pacini corpuscles (Pa.C.) has been demonstrated previously by Barbolini, Tischendorf & Curri (1971). The glomic AVA are highly specialized devices for the regulation of the capillary blood flow (Clara, 1939) and undergo several alterations in peripheral vascular diseases and other pathological conditions, such as diabetes, cirrhosis, hypertension and atherosclerosis (Curri, 1971, 1973).

It has also been observed that physiological senile involution of the AVA is frequently associated with regressive changes of the Pa.C. (Barbolini, Tischendorf & Curri, 1972/1973) with fusion of the lamellae and narrowing, fibrosis and homogenization of their structure. To date it has not been ascertained if primary changes of the Pa.C. are able to provoke microcirculatory changes due to abnormal shunting through the AVA. The case of Pacinian neurofibroma here reported gave the opportunity to verify this hypothesis on the basis of clinical and morpho-histochemical observations. Bennin, Barsky & Salgia (1976) have reviewed the few cases of Pacinian neurofibroma described in the literature; it is a benign skin tumour whose characteristics are summarized in Table 1.

Our case of Pacinian neurofibroma involving the whole of the left ring finger is different from those reported in the literature for its extent, for the intensity of the pain and for the Raynaud-like symptoms.

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TABLE 1. Clinical and histological features of the reported cases of Pacinian neurofibroma

| Authors | Site | Clinical characteristics | Histology |
|---|---|---|---|
| Prichard & Custer (1952) | Hand: dorsal metacarpal region | Button-like, painless nodule | Encapsulated mass |
| Prichard & Custer (1952) | Buttock | Painless nodule | Partially encapsulated mass |
| Prose, Gherardo & Coblenz (1957) | Heel | Partially ulcerated, well delimited tumour | Encapsulated mass |
| Bennin *et al.* (1976) | Index finger tip | Round, firm, well marginated nodule | Not encapsulated mass |
| MacDonald & Wilson Jones (1977) | Dorsum of thumb | Domed, smooth, mobile nodule | Encapsulated mass |
| | Neck | Raised, pigmented, mobile nodule | Encapsulated mass |
| | Hand: dorsum 3rd metacarpophalangeal joint | Domed, slightly pedunculated nodule | Encapsulated mass |
| | Hand: extensor surface thumb | Smooth, papular, soft lesion | Encapsulated mass |

## CASE REPORT

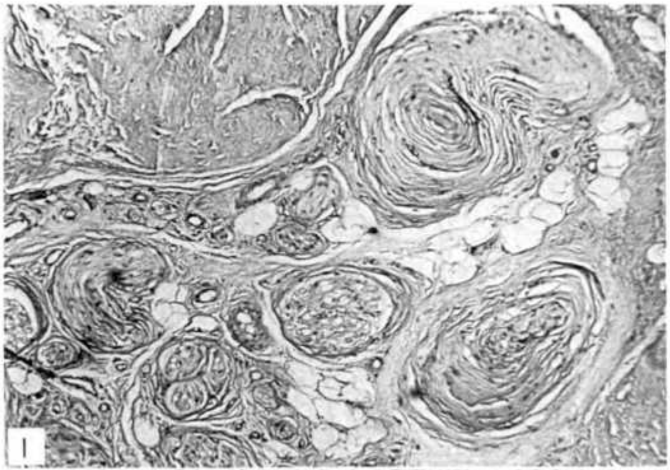
B.R., a 60-year-old woman, complained of pain in all the fingers with Raynaud-like symptoms from the age 21 years. In the past 2 years the pain had become very intense and persistent especially along the ring finger of the left hand, and was not amenable to any treatment. There was no evidence of swelling of the finger or other inflammatory symptoms. Because of persistence of intense pain it was necessary to amputate the ring finger, with complete disappearance of the pain, but without substantial modification of the Raynaud-like symptoms in the other fingers. The whole finger was examined histologically with serial sections of the three phalanges.

### *Microscopy*

The serial sections were stained with H & E, Alcian blue, P.A.S. and Masson's trichrome stains. The microscopic examination showed a similar picture in the three phalanges. The epidermis shows marked orthokeratosis with a normal granular layer. The Malpighian layer is in some areas hypertrophic and in others atrophic. There are occasional dilated vessels in the papillary dermis. In the deeper dermis there are numerous round or ovoid corpuscles composed of concentric, onion-like lamellae delimited by a fibrous capsule (Fig. 1).

The size of these bodies is very variable and their morphology varies according to their stage of maturation. The more immature stage has many cellular elements with spindle-shaped nuclei and loose or dense chromatin (Fig. 2). These cellular bodies show a whorling appearance about a central area corresponding to the 'stalk' and are embedded in a weakly eosinophilic substance; the initial phases of a lamellar differentation can be observed. In the next stage, the nuclei become less dense in the middle and little vacuoles appear which fuse to form circular or ovoid spaces delimited by a thin faintly eosinophilic membrane (Fig. 2).

In some corpuscles it is still possible to note cellular fragments. At the periphery two or three layers of the lamellae can be observed, formed by long, thin spindle cells. In some of these corpuscles it is easy to observe a capillary, always in a central location. In the third stage the core of the corpuscle



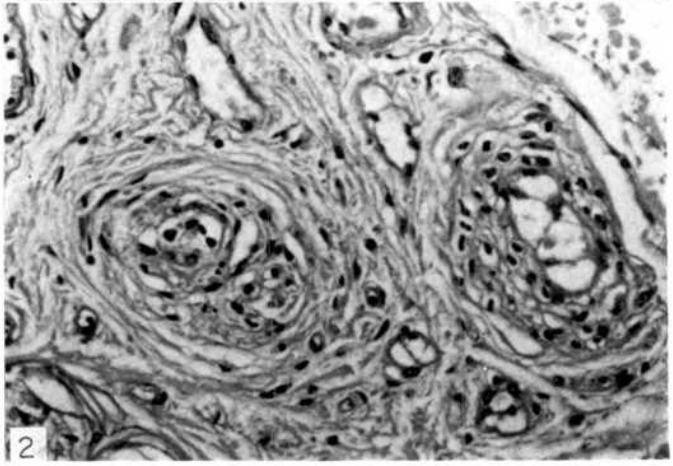


FIGURE 1. Newly formed, irregularly distributed Pacinian corpuscles in various stages of maturation, composed of concentric onion-like lamellae and located in the deeper dermis (Alcian blue-PAS stain, × 120).

FIGURE 2. Proliferation and differentation of dysplastic Pacinian corpuscles: in this stage of the lamellar differentation, little vacuoles appear fusing to form circular or ovoid spaces delimited by an eosinophilic and PAS-positive membrane (Alcian blue-PAS stain, × 200).

subdivides into round septate areas and, in the more mature stage, the histology is that of a mature Pacinian corpuscle.

In the finger-tip sections abnormal encapsulated AVA of glomus type are present. The intermediary segment shows a dissociated media with atrophy of the media and with degenerative changes in the myoepithelioid cells and an outer thick layer of concentric fibrosis. The lumen of the AVA is large and open ('hyperstomy') (Curri, 1973). Also the media of the afferent arterial system is distorted whereas the collecting venules are abnormally dilated (Fig. 3).

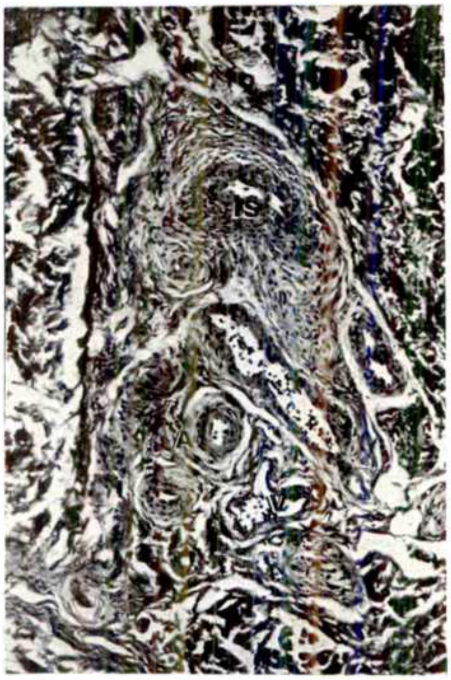


FIGURE 3. Arterio-venous anastomosis of the glomus type of the finger tip. The media of the intermediary segment (IS) shows centripetal sclerosis with dissociation of the myoepithelioid cells; the arterial afferent system (A) is distorted and the collecting venules are abnormally dilated (V) (Masson's trichrome stain, × 100).

## DISCUSSION

Our case is similar in its location to that reported by Bennin *et al.* (1976). It differs in that the tumour is not solitary and more or less encapsulated, but has the characteristics of a real dysplasia involving the whole finger, with a number of newly formed Pa.C. in different stages of development. It is probable that the massive proliferation of these nervous structures was responsible for the burning pain, not referred to in the previously reported cases.

Clara (1939) postulated that the Pa.C. are regulators of blood flow because of their topographic connection with the finger-tip AVA: the nervous impulse arising from the corpuscles provokes a vasomotor reflex with consequent opening of the shunt. The new formation of Pa.C. and the aberrant nervous impulses may be responsible for pathological changes in the AVA ('hyperstomy'), with clinical repercussions, such as the Raynaud-like symptoms. The close relationship between the altered shunting function of the AVA and the Raynaud's syndrome has been described (Tischendorf & Curri, 1963); in our case the morphological alterations of the AVA vessels are even more pronounced because of either the Raynaud's syndrome or the nervous stimuli arising from the Pacinian neurofibroma.

On the basis of the histological and clinical findings the term 'Pacinian neurofibromatosis' is proposed, to differentiate the dysplastic proliferation of a great number of Pa.C in the whole ring finger from the single, painless Pacinian neurofibroma without clinical signs of peripheral vascular disease.

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## *Brief Communications*

### Bone formation in a cutaneous pyogenic granuloma

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Bone formation in angiomatous naevi such as strawberry naevi is recorded (Rook, 1972) but as far as we are aware ossification in a cutaneous pyogenic granuloma has not been reported.

### CASE REPORT

A 19-year-old student nurse presented to this department with a 6-month history of a red, raised lesion on her left palm which blanched on pressure. There was no definite history of trauma at the site. On examination she had a 5 mm diameter, red, crusted, dome-shaped lesion which clinically looked like a superficially ulcerated pyogenic granuloma. The lesion was removed in one piece by curettage.

Histology showed a circumscribed vascular lesion composed of dilated capillaries surrounded by fibroblasts and an excess of endothelial cells. There was ulceration with an acute inflammatory exudate at the superficial margin. Deeper in the lesion were strands of metaplastic calcification with

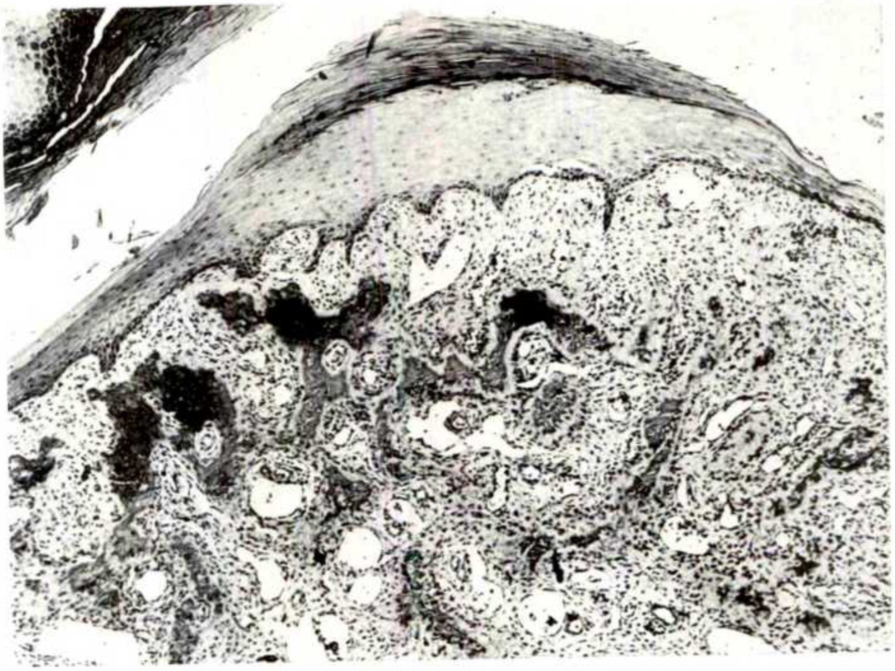

FIGURE 1 Pyogenic granuloma showing metaplastic calcification and ossification (H & E, × 90).

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areas of ossification (Fig. 1); this feature was thought to be unusual in an otherwise typical pyogenic granuloma.

## DISCUSSION

Ossification can occur in capillary angiomata and has been reported in pyogenic granuloma of the oral cavity (Bhaskar & Jacoway, 1966). Pyogenic granuloma is a relatively common benign vascular tumour and is believed to occur with equal frequency on the skin and mucous membranes (Berlin, Block & Donick, 1972). It is therefore surprising that ossification in pyogenic granuloma of the skin has not been previously observed.

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# Syndrome of ichthyosis, hepatosplenomegaly and cerebellar degeneration—steroid sulphatase activity

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We recently reported two brothers with ichthyosis, hepatosplenomegaly and cerebellar degeneration (Dykes, Marks & Harper, 1979). Analysis of pedigree suggested a recessive or possibly X-linked mode of inheritance. The occurrence of skin changes in an otherwise normal brother indicated the possibility of the presence of two discrete disorders such as X-linked ichthyosis and an autosomal recessive storage disorder. This was considered unlikely as the histopathological and electron microscopical features of the ichthyosis were not characteristic of X-linked ichthyosis. Shapiro *et al.* (1979) have demonstrated that steroid sulphatase activity is absent in the cultured fibroblasts derived from patients with X-linked ichthyosis. We wish to report the results of assays performed on fibroblasts derived from these two brothers.

Fibroblast cultures were obtained from skin biopsies using conventional techniques. Confluent cultures were assayed for steroid sulphatase activity using dehydro-[7(n)–$^3$]Hepiandrosterone sulphate (The Radiochemical Centre, Amersham) as substrate. Cells were harvested by trypsinization and, after disruption by freeze-thawing, homogenized in a small glass homogenizer in 0·05 M Tris-HCl, pH 8·0 buffer containing 0·1 mg/ml sodium dodecyl sulphate. Reaction mixture (final volume = 0·35 ml) consisted of homogenate, buffer and substrate at a final concentration of 0·32 $\mu$M (specific activity = 4·6 Ci/mmol). After incubation at 37°C for 2 h the reaction was stopped by adding 0·35 ml methanol. The desulphated dehydroepiandrosterone was extracted with hexane and the radioactivity measured by scintillation counting. The release of radioactivity was linear with time over the 2 h period. Appropriate controls consisting of buffer plus substrate were incubated in parallel.

TABLE 1.

| Subject | pmol converted per hour per mg protein |
|---|---|
| Case 1 (C.G.) | 3·53 |
| Case 2 (R.G.) | 6·61 |
| Normal 1 | 3·47 |
| Normal 2 | 2.03 |
| Ichthyosiform erythroderma | 2.26 |
| X-linked ichthyosis | —* |

* Release of $^3$H not above background.

The results are presented in Table 1. Fibroblasts derived from both cases showed activity comparable to that shown by cells derived from two normal individuals and a patient with ichthyosiform erythroderma. Cells derived from a patient diagnosed as having X-linked ichthyosis on clinical and histopathological grounds had no measurable activity. These observations indicate that it would be a

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remarkable coincidence indeed if two discrete disorders were present in this family. It seems most likely that these brothers show manifestations of an as yet unidentified metabolic defect with multisystem involvement similar to that seen in Refsum's disease.

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***Society Proceedings***

# Eastern Counties Dermatological Society
# March 1979

## ADDENBROOKE'S HOSPITAL, CAMBRIDGE

### Urticarial vasculitis and IgA myeloma

DR ALLAN S. HIGHET

A man, aged 44 years.

*History.* This man presented with a 4-year history of symptomless weal-like lesions on the arms and trunk. The lesions each lasted for several days and were not improved by antihistamines.

*Investigations.* Serum complement studies showed intermittent reduction in total haemolytic complement activity (e.g. approximately 600 u/ml; normal range 900–1800 u/ml), and high levels of immune complexes as assessed by the method of Harkiss & Brown (1979) (e.g. 100%, 44%, 48%; normal range 0–24%). C3 levels were repeatedly normal. Serum activities of Cl esterase inhibitor, factor B, and total alternative pathway were not reduced.

Cryoglobulin was not detected. Auto-antibodies, including anti-nuclear antibody, were not detected.

An infective source of antigen was not found. Prothrombin time, kaolin partial thromboplastin time, and thrombin time were normal.

A biopsy of a 10-day-old lesion showed an acute vasculitis with polymorphs predominating. Immunofluorescence showed fibrin and IgA in a pattern suggesting extravasation into the dermis but was negative for IgG, IgM, Clq, and C3.

Lethargy, skeletal pains, hepatosplenomegaly, and an ESR of 100 mm in the first hour prompted haematological investigation leading to a diagnosis by the general physicians of IgA myeloma. The patient had widespread osteolytic lesions but had normal renal function. The IgA had lambda light chains and the serum concentration was 6 g/l (normal range 1–3·5 g/l). Serum levels of both IgG at 3·0 g/l (normal range 7–13 g/l) and IgM at 0·2 g/l (normal range 0·5–2·0 g/l) were reduced. A rectal biopsy showed no evidence of amyloid, but scanty perivascular IgA was noted.

*Treatment.* Three-weekly 5-day courses of prednisone 40 mg daily and melphalan 5 mg daily were started. The skin lesions cleared by the end of each course but recurred after about 10 days. A maintenance dose of prednisone 10 mg daily between courses prevented these recurrences. The serum concentration of paraprotein showed no consistent change throughout a treatment cycle.

#### DISCUSSION

These atypical weals with a vasculitic histology may be described as 'urticarial vasculitis' (Gammon & Wheeler, 1979). A frequent finding in such cases is hypocomplementaemia with evidence of circulating immune complexes, as in the present case. This patient also had IgA myeloma.

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### *Cutaneous vascular disease associated with IgA paraproteinaemia (including myeloma)*

The association of urticarial vasculitis with IgA paraproteinaemia does not seem to have been previously reported.

The strongest dermatological association with IgA paraproteinaemia is pyoderma gangrenosum (Holt, Davies & Nuki, 1978; Möller, Waldenström & Zettervall, 1978). A patient described by Thompson *et al.* (1973) had leucocytoclastic vasculitis, pyoderma gangrenosum and IgA paraproteinaemia. Histological overlap between leucocytoclastic vasculitis and pyoderma gangrenosum is mentioned by Ryan (1976a). IgG and IgM paraproteinaemias are less commonly associated with pyoderma gangrenosum.

There are several reports of erythema elevatum diutinum ( a form of leucocytoclastic vasculitis) in patients with paraproteinaemia, especially of IgA type (Archimandritis *et al.*, 1977; Katz *et al.*, 1977; Kövary, Dhonau & Happle, 1977).

IgA paraproteinaemia has also been recorded in association with erythema nodosum (Meiers, Orfanos & Schilling, 1967) and with angio-oedema (Cheesbrough & Kinmont, 1978). Valdes *et al.* (1976) found histological evidence of dermal microangiopathy in patients with IgA and IgG myelomas and macroglobulinaemia. Vessel wall infiltration and cutaneous haemorrhages occur in systemic amyloidosis (Bleehen, 1979), which may complicate paraproteinaemia of any immunoglobulin type (Isobe & Osserman, 1974).

### *Possible mechanisms in skin disease associated with paraproteinaemias*

Whether these forms of cutaneous vascular disease can be directly attributed to the accompanying paraprotein, or to an associated underlying condition (such as collagen disease or, as in this case, neoplasia) is uncertain. Possible mechanisms remain speculative, as with the present patient.

Hyperviscosity might be invoked, but this is rare in IgA paraproteinaemia (Thompson, 1977). Blood viscosity was not measured in the present case.

Wallington & Reeves (1978), studying a case of vasculitis with macroglobulinaemia, rheumatoid disease and cryoglobulinaemia, suspected complement fixation by cryoglobulins in vessel walls, but cryoglobulins were not detected in the present patient.

IgA may activate the alternative complement pathway (Muller-Eberhard, 1976), but this would not account for the presence of circulating immune complexes in our patient, and his alternative pathway studies were normal.

Neoplasms, including leukaemias, can increase intravascular coagulation which may play a part in vasculitis (Ryan, 1976b), but in the present case routine coagulation studies were normal.

Metabolic factors and drug effects, in addition to immunological and coagulation abnormalities, were suggested by Valdes *et al.* (1976) as possible bases for myeloma-associated microangiopathy.

Low serum activity of Cl esterase inhibitor has been reported: (a) in the patient of Cheesbrough & Kinmont (1978) with angio-oedema and IgA paraproteinaemia; (b) in a patient with cold urticaria, lymphosarcoma and IgA monoclonal cryoglobulinaemia (Hauptmann *et al.*, 1975); and (c) in a patient with Raynaud's phenomenon, cold agglutinin disease, and IgM paraproteinaemia (Wallington, French & Reeves, 1978). The pathogenetic significance of these findings is not clear. The present patient, however, had normal Cl esterase inhibitor activity.

It is increasingly recognized that circulating immune complexes which fix complement in vessel walls are involved in many cases of leucocytoclastic vasculitis (Melski & Soter, 1979). The present patient had high levels of circulating complexes and it is possible that these were involved in the skin lesions. However, the nature of the association of the immune complexes with the myeloma is not clear; the extravasation of IgA was probably a non-specific effect of increased vessel permeability. Möller *et al.* (1978), in the context of pyoderma gangrenosum with IgA paraproteinaemia, suggested that the depression of the normal immunoglobulins might predispose to infection; in the present case, however, no infective source of antigen was discovered. IgA does not fix complement and is not likely to have been acting as an antibody in the causation of the skin lesions. If the IgA were itself antigenic and complexed with an IgG or IgM antibody, complement fixation could occur. This possible involvement of an anti-

IgA antibody has not been ruled out in the present case, although the absence of cryoglobulins is a point against it, since most such complexes would precipitate in the cold. Tumour antigens have been held responsible in cases of immune complex vasculitis associated with lymphoproliferative disorders (Melski & Soter, 1979). This mechanism would be very difficult to confirm or refute, and remains a possibility in the present patient.

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## Urticarial vasculitis resembling systemic lupus erythematosus: efficacy of prednisone and dapsone combined.

Dr Allan S. Highet

A man, aged 44 years.

*History*. This builder developed weal-like lesions in January 1977. Vasculitis rather than ordinary urticaria was suggested by the longer duration of the lesions (each lasting several days), the symptom

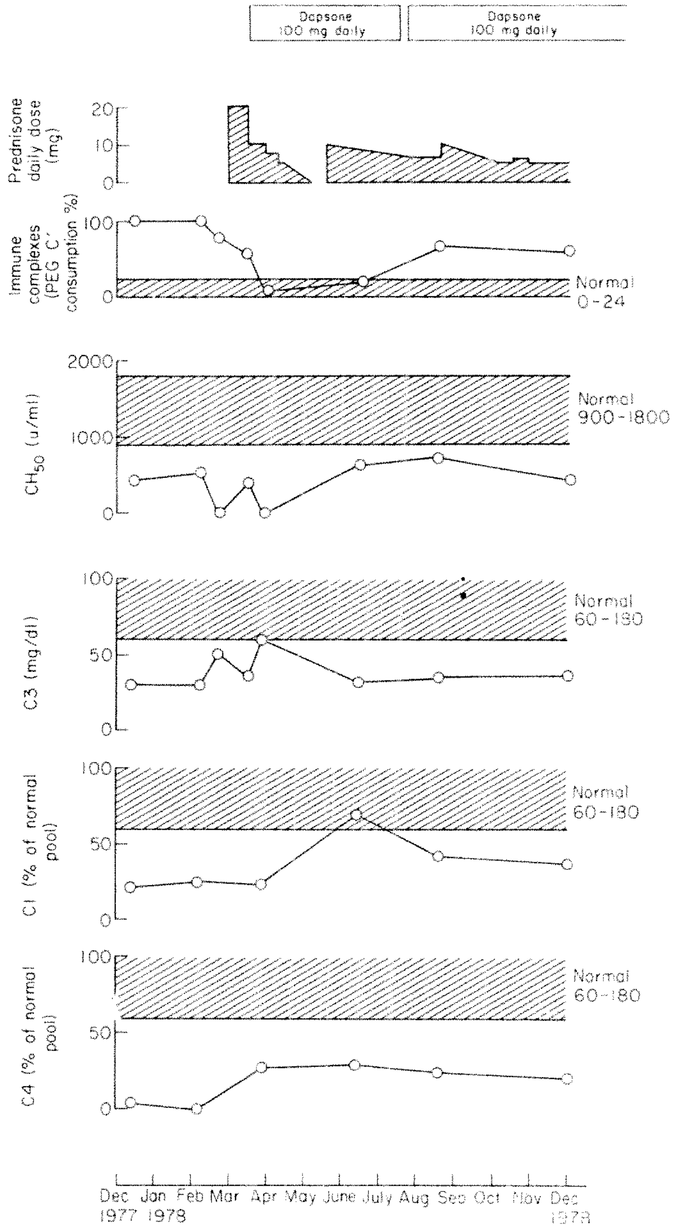

FIGURE 1. Changes in complement components and immune complexes in serum with treatment.

of slight pain rather than itch, and a disappointing response to antihistamines. He later developed joint pains, pleuritic chest pain, blotchy erythema, photosensitivity and purpura.

*Investigations.* The histology of a 3-day-old lesion showed a moderately severe leucocytoclastic vasculitis. Both lesional and non-lesional skin had positive immunofluorescence (for IgG, IgM, Clq, and C3, granular pattern) at the dermo-epidermal junction.

The serum showed a marked reduction in total haemolytic complement activity ($CH_{50}$) and in the complement components C1, C2, C3, and C4. There were high levels of immune complexes as measured by the method of Harkiss & Brown (1979) (Fig. 1). C6, C7, Factor B, and total alternative pathway activity were normal. These findings are typical of immune complex activation of the classical complement pathway.

Mixed cryoglobulins were present. The haemoglobin was slightly reduced at 12–13 g/dl. The erythrocyte sedimentation rate was slightly raised at 20–33 mm in the first hour. Renal function was normal. Tests for anti-nuclear antibody and smooth muscle antibody were occasionally positive, but only in insignificant titres of 1 in 25 or less. DNA binding was repeatedly normal. A search for other antigens proved negative.

*Treatment.* On prednisone 20 mg daily the patient made slight clinical improvement but the serum level of immune complexes fell to normal and there was marked improvement in the C3, C1, and C4 levels. The addition of dapsone 100 mg daily led to marked clinical improvement and the dose of prednisone could be reduced to between 5 mg and 10 mg daily without clinical deterioration. However, the immune complex level then rose again and the complement levels fell. The contribution of dapsone was confirmed by the recurrence of weal-like lesions and malaise within 5 days of its withdrawal; these features responded rapidly to re-introduction of the drug.

## DISCUSSION

The clinical and laboratory findings are consistent with an immune complex disease, but the source of antigen was not discovered.

Recent reports of similar cases have been reviewed by Gammon & Wheeler (1979). They regarded the essential feature of this 'urticarial vasculitis' or 'unusual SLE-related syndrome' as the association of weals (clinically often not typical of ordinary urticaria) with the histological picture of leucocytoclastic vasculitis. Different aspects have been investigated by different authors, but common additional features include: angio-oedema, other cutaneous manifestations of vasculitis, arthralgia or arthritis, elevated ESR, hypocomplementaemia, evidence of circulating immune complexes, and immunoglobulins and complement at the basement membrane zone in lesional skin and, where reported, in non-lesional skin. Pyrexia, abdominal symptoms, and renal disease have been reported.

This syndrome may vary in severity from mild, resembling ordinary urticaria, to severe, resembling systemic lupus erythematosus (SLE); Gammon & Wheeler (1979) felt that it could be differentiated from SLE itself, especially by failure to meet the diagnostic criteria of the American Rheumatism Association (ARA), by absence of antibodies to double-stranded DNA, and by either the absence of or only mild renal disease. However, even this distinction may be blurred, as shown by the present patient whose condition does meet the ARA criteria for SLE (Hughes, 1977); and it is noteworthy that not all twelve cases of 'chronic urticaria-like lesions in SLE' reported by O'Loughlin, Schroeter & Jordan (1978) had positive anti-nuclear antibody. Completely satisfactory criteria for SLE are lacking and it is debatable whether the characteristic anti-nuclear and anti-DNA antibodies should be regarded as absolutely necessary for the diagnosis (Hughes, 1977).

The effects of prednisone and dapsone are interesting. The effects of corticosteroids on inflammatory processes are not fully understood, but include (a) suppression of inflammatory cell and mediator activity at the site of tissue damage, and (b) reduction of antibody production at higher doses (Snell, 1976). The dramatic fall in levels of immune complexes seen in the present case on a daily dose of prednisone of 20 mg but not of 10 mg or less might be due to reduced antibody production.

Prednisone might also have had a local anti-inflammatory effect, but clinical improvement was greatly enhanced by the addition of dapsone. The possible modes of action of dapsone in inflammatory diseases are discussed by Alexander (1975), Katz *et al.* (1977), and Stendahl, Molin & Dahlgren (1978). All the evidence points to local effects at the site of inflammation, perhaps including inhibition of the alternative complement pathway and, probably more relevant in the present case, inhibition of the myeloperoxidase system of polymorphonuclear leukocytes. Matthews, Saihan & Warin (1978) referred to the use of dapsone in vasculitis and reported a case, similar to the present one, in which the skin lesions responded well to dapsone alone.

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## *Comment*

# CLINICAL AND LABORATORY CRITERIA FOR THE DIAGNOSIS OF BEHÇET'S DISEASE

In 1937 Behçet described the triple symptom-complex of recurrent orogenital ulceration and eye inflammation. It has become apparent now that the process is a systemic disease affecting a number of organ systems (Berlin, 1960; Haim, 1968). The original three cardinal symptoms of this malady are often separated by years and its remaining manifestations are difficult to delineate clearly from other systemic disorders on clinical grounds alone. This results in considerable controversy regarding the clinical criteria demanded to establish a firm diagnosis of Behçet's disease. If we hold the original triple symptom complex a prerequisite for the diagnosis, cases may be missed and liberalization of these criteria may include other entities (O'Duffy, Corney & Deodhar, 1971; O'Duffy, 1972; Chajek & Fainaru, 1975). In spite of these facts the currently accepted criteria for the diagnosis of Behçet's disease remain based on clinical grounds alone. Curth (1946) suggested that only two manifestations out of the original triad of symptoms, i.e. oral and genital ulceration and eye inflammation, are necessary for diagnosis. Mason & Barnes (1969) constructed an elaborate set of major and minor criteria for the diagnosis. They suggested buccal ulceration, genital ulceration, eye lesions and skin lesions as major symptoms. The set of their minor symptoms included gastro-intestinal lesions, thrombophlebitis, cardiovascular lesions, arthritis, C.N.S. lesions and family history. According to the authors a minimum of three of the major, or two major and two minor, criteria are required for the diagnosis. O'Duffy (1978) suggested that the diagnosis of Behçet's disease can be established when repeated oral aphthous ulcers occur in combination with only two of the following additional features: genital ulceration, uveitis, synovitis, cutaneous vasculitis and meningo-encephalitis. There is not a single criterion among all the above mentioned symptoms which is sufficiently specific to establish the diagnosis. Each one of them is met with alone or in combination with others in a variety of disorders. All the above manifestations may accompany granulomatous colitis (O'Duffy, 1978). The association of ulcerative colitis with aphthous ulceration, erythema nodosum and arthralgia is well documented. So, basing the diagnosis only on clinical symptoms demands some rider to prevent false positive categorization of the disease. The question arises whether we can incorporate non-clinical features in the diagnostic criteria at least in some doubtful cases.

Studies have revealed various immunological and other laboratory findings which proved to be characteristic, though not necessarily pathognomonic, of this malady. Among these findings is the skin hyperreactivity (pathergy) which has long been utilized in the diagnosis of our cases (Haim & Sherf, 1966; Haim, 1968; Haim, Sobel & Friedman-Birnbaum, 1974). This phenomenon was first described by Blobner (1937) and Jenson (1941) and was further elaborated by Katzenellenbogen (1968) who regarded it as being pathognomonic of this disease in that it was absent in control groups with recurrent aphthous stomatitis and with systemic lupus erythematosus. In a recent study conducted by Tüzün *et al.* (1978), this test was found positive in 84% of fifty-eight patients with the disease as compared to 3% of ninety healthy and diseased controls including recurrent aphthous stomatitis, iridocyclitis, erythema nodosum, rheumatoid arthritis, Reiter's syndrome, etc. The diagnostic value of this sign in our practice is well illustrated by the following case. The patient was referred because of superficial recurrent thrombophlebitis of the lower extremities. During a period of 6 months, the clinical and laboratory investigations were essentially not contributory apart from a positive pathergic

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test which kept us aware of the possibility of Behçet's disease. The diagnosis of the disease was well established when, at the end of this period, the patient started suffering from recurrent ulceration of the oral mucosa and genitalia in addition to the thrombophlebitis (Haim *et al.*, 1974). From our experience, a positive pathergic reaction makes us aware of the possibility of the disease in the presence of any of the accepted symptoms of this process, including recurrent oral ulceration alone. The criteria for the diagnosis of our cases, so far, are based on at least two of the three cardinal and one of the other manifestations recognized as a complication of the disease. Besides the clinical symptoms we also consider the pathergic reaction and the recurrent nature of the symptoms in our diagnosis (Haim & Sherf, 1966; Haim, 1968).

Apart from the pathergy various immunological and laboratory findings are accepted today as characteristic though not pathognomonic of this malady such as: increased cell-mediated immunity (Lehner, 1972; Haim *et al.*, 1976a); enhanced chemotactic activity of the polymorphonuclear cells (Matsumura & Mizushima, 1975; Sobel *et al.*, 1977), specific histocompatibility antigen (Ohno *et al.*, 1975; Haim, Gideonik & Barzilai, 1977); elevation of serum C9 levels (Kawachi-Takahashi *et al.*, 1974); frequent positive intradermal test with streptococcal antigen (Yamada & Yanase, 1977); and significant increase of degranulated mast-cells in the mucocutaneous lesions (Lehner, 1969; Haim *et al.*, 1976b). Yet none of the diagnostic criteria so far suggested consider these findings. This raises another aspect of the diagnostic problem, that is the question of the diagnosis of cases which are clinically consistent with Behçet's disease and at the same time lack all these findings, as was demonstrated in one of our cases. This case presented recurrent oral and genital ulceration as well as superficial thrombophlebitis. However, the cell mediated immunity and his skin response to various intradermal tests were obviously depressed. We are still investigating a possible underlying cause for the depressed immunity and the case is not yet added to our series with the disease.

We feel that the question of the diagnosis of Behçet's disease requires careful thought and that clinical as well as laboratory findings must be considered in this respect. After all, what was originally described as a syndrome is now universally recognized as a disease.

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*British Journal of Dermatology* (1980) **102,** 365.

# *Correspondence*

## Dapsone-induced haemolytic anaemia

SIR, I was interested to read the article by Dr Giuliano Manfredi and his colleagues (1979) connected with dapsone-induced haemolytic anaemia. Particularly important is their conclusion that 'haemolytic anaemia in normal G-6-PD bearing subjects during dapsone treatment is not due to an impairment of the G-6-PD system'.

The problem of the relationship between G-6-PD deficiency and dapsone has been perturbing workers for quite a time. In 1964 I published an account of a survey of over 1000 cases of Hansen's disease of whom we found 4·4% to be G-6-PD deficient. All patients were taking between 600 and 800 mg of sulphone per week and we found that there was no patient with a frank haemolytic anaemia nor were signs of haemolysis noticeable in the plasma of any of these cases.

Plewig & Kligman (1975) suggested that 'sulphone, perhaps because of its well-publicised toxicity, still seems to be a little beyond the pale (but) it is a powerful and magnificent agent'.

I would therefore urge dermatologists who are considering the use of sulphone not to be too frightened by the possibility of dapsone induced haemolysis and suggest that there is probably little indication to look for G-6-PD deficiency in their potential patients before initiating sulphone therapy.

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### REFERENCES

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SIR, Dr Pettit's comments are very interesting and relate to practical problems we face every time we prescribe dapsone. Our experience is not comparable to the enormous experience of Dr Pettit; however we maintain that patients receiving large amounts of dapsone, for example, in our cases 1400 mg weekly, inevitably develop methaemoglobinaemia and subsequent haemolysis. In our paper we demonstrated (Fig. 2) that there is a strict correlation between dapsone dosage and methaemoglobinaemia. In fact, as reported in Table 1 of our article, after 1 week of dapsone treatment, 100 mg/day, we obtained a methaemoglobinaemia percentage of 5·40 ± 0·44 which is well tolerated. When increasing the doses, we systematically observed an increase of the methaemoglobinaemia and, concomitantly, the appearance of the symptoms associated with this drug. The good tolerance to this drug in leprosy patients could be explained as due to the relatively low dosage of dapsone (100 mg/day) usually employed in these patients, as reported at the 11th International Congress of Leprosy (1979).

Unfortunately and in spite of the optimistic considerations of Plewig & Kligman, we maintain that dapsone, though powerful, does remain an awkward drug to manage when given in *high doses* as recommended for the treatment of dermatitis herpetiformis (Fitzpatrick *et al.*, 1979).

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## Progressive melanosis of the skin

Sir, The case reported in detail by Dr Levene (*British Journal of Dermatology* (1979) **101**, 197) may be identical with one or more of those described by Darier in 1925 and referred to in his *Precis* (Darier, 1928) as mesenchymatous malignant melanoma or melanosarcoma or progressive melanosis. Darier comments on the similarity to the melanosis of white horses.

The case I reported (Bettley, 1938) more closely resembles those subsequently described by Ito. In my paper I included a brief account of a calf affected by benign melanosis and accepted Darier's suggestion that the human cases have much in common with those of horses and cattle. My case showed no evidence of dissemination at the time, but was lost to follow-up.

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REFERENCES

Bettley, F.R. (1938) Progressive Melanosis of the Skin. *British Journal of Dermatology*, **50**, 181.
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* * *

Sir, I thank Dr Bettley for drawing my attention to references I had not encountered in my reading.

Darier's statement 'It is more than likely that certain melanomas of choroidal origin belong to this [blue-naevus] category. This is true also, as far as I am able to ascertain, for the melanosis of white horses.' This is a remarkable statement for the time when the French literature on equine melanotic disease lacked all reference to such a concept. Malignancy supervening on equine melanotic disease is a relatively uncommon outcome in a disorder affecting 90% of a specific ageing population and, as I have made very clear, does not usually appear to be malignant change in a pre-existing visible tumour. This casts some doubt, I believe, on the significance of Darier's statement.

Dr Bettley, who preceded Ito in his report of an extra-sacral Mongolian patch on the shoulder region, showed that the condition is a simple dermal melanocytosis, not differing histologically in appearance from the sacral variety. The major differences between the naevus of Bettley/Ito and the present case reside in two clinical factors:

(1) Though malignant change in naevus of Ota has been recorded in non-Japanese only (Dorsey & Montgomery, 1954; Mishima & Mevorah, 1961), there are no such reports in the case of naevus of Ito.

(2) When malignancy does arise with naevus of Ota or in a blue naevus, there is obvious change *in situ*. In the present case, if there was a primary tumour arising in the dermal melanocytosis, it remained hidden.

After my paper went to press, I found that in 1951 Findlay & Rand described two cases of mesodermal melanosis of the face and sclera with abnormalities of the cranium. Histologically the appearances were those of dermal melanocytosis and the cranial anomalies, as they pointed out, resembled those described by Carleton and Biggs in the present case. Briefly, in one case there was a prominent knob at the outer part of the superior nuchal line at each side giving the occiput a somewhat square appearance and in the other there was a conspicious prominence on the cranial vault below the lambda where the skull contour was notched by a particularly projecting occiput, with other rounded prominences of fronta land other flat bones of the skull. There was no follow-up of these two children.

Some assistance in understanding this curious combination of abnormalities comes from an appreciation of the versatility of neural crest cells and their capacity to migrate and differentiate into several varieties of tissue. Weston (1970) in his detailed review of neural crest development points out that apart from giving rise to pigment cells, it provides a major contribution to the cranial and visceral skeleton of the head and neck.

It seems reasonable now to make the following additional suggestions about this case:

(1) That Carleton and Biggs first described a new syndrome consisting of cranial deformity associated with an anomalous cutaneous pigment system.

(2) That the syndrome is an example of a neural crest anomaly.

(3) That the frequently multicentric presentation of equine melanotic disease, especially in its superior nuchal and parotid distribution, is also suggestive of a neurocristic anomaly.

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*British Journal of Dermatology* (1980) **102,** 368.

# *Book Reviews*

**Dermatosurgery.** J. PETRES and M.HUNDEIKER (1978) New York: Springer-Verlag. Pp. 152, 112 illustrations.

This book which sets out to bridge the gap between dermatology and surgery is translated from the original German. The text is idiomatic and easy to read and the main evidence of its origin is in the extensive bibliography. It is a little difficult to be sure for whom the authors were writing the book. The opening thirty pages are highly condensed clinical details of those skin disorders which may be treated surgically and are superfluous for dermatologists. This section is presumably included for the benefit of those who operate from a non-dermatological background. The remainder of the book is divided into a smaller section on the principles and technique of skin surgery and a larger section in which are described the operative techniques employed in the different regions of the body. These sections are illustrated by numerous clear line drawings and by a series of monochrome plates at the end of the book. It was evidently not the authors' intention to write a detailed, step-by-step manual of operative surgery, and one feels that this section is perhaps inadequate for the needs of the operator whether he comes from a surgical or dermatological background. Another minor shortcoming is the rather too partisan advocacy of surgery as the treatment of choice. The value of radiotherapy is underrated and rather little attention is paid to the economic implication of the various procedures. The authors succeed in their aim of providing a simplified account of the various surgical and reconstructive procedures employed for skin disorders and the book can be recommended to anyone who needs to advise patients on the range of operations that can be performed. The quality of illustration and production cannot be faulted.

K.V.SANDERSON

**Principles and Practice of Dermatology.** WALLACE E. DE LAUNEY and WILLIAM A.LAND (1978) Sydney: Butterworths. Pp. 294, 44 illustrations.

Relatively few books on dermatology reach this country from Australia and those which come to mind have dealt with skin malignancy. This modest book, written for the Australian undergraduate and general practitioners, would serve equally well as an introduction or guide in this country. It is traditional in its approach and layout, written from the clinician's viewpoint with the bare minimum of scientific background. Its strength is its common-sense approach to practice expressed clearly and simply. There are a small number of line drawings and a rather larger number of small colour illustrations grouped in eight plates in the centre of the book, which are of variable quality. It is here, perhaps, that the authors' claim to give special emphasis to tumours is best shown but even so the illustrations of malignant melanoma are disappointing. An increase in the quality and scope of the colour illustrations should be the first priority for a second edition. In other respects the quality of production of the book is in the good 'economy' class. One may question the relative weight given to different diseases (leprosy gets less attention than it merits, for instance, for a country which abuts S.E. Asia) and one may disagree with some details, e.g. radiotherapy for acne. In general, however, this is a worthy contender in the undergraduate textbook market and would not be out of place on the general practitioner's bookshelf.

K.V.SANDERSON

**A Compilation of Journal Instructions to Authors.** K. ROBERT MCINTIRE (1979) U.S. Department of Health, Education, and Welfare Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication, No.79.1991, June 1979.

Dr McIntire has collected together the Instructions to Authors from 290 different medical and scientific journals. It seems unlikely that uniformity in journal style will be achieved in the immediate future. Authors (or their secretaries) who favour several journals with their writings may find access to this book of some value, even though virtually all the individual journals do themselves carry this information, and dermatology is rather under-represented.

R.H.Champion

**Year Book of Dermatology 1978.** Edited by F.D.Malkinson and R.W.Pearson (1979) Chicago: Year Book Medical Publishers. Pp. 383. Price £19.75.

The Year Book follows its usual format containing sixteen sections each covering an aspect of Dermatology. In each section papers published up to December 1977 are summarized, and this is done fully yet concisely; each precis is often followed by a short critical examination of it, related papers are discussed and the authors' own clinical experience used to put the results and views expressed into perspective. This is most helpful. Each section ends with references to further recommended reading. A review article of the connective tissue diseases opens the book; in this thirteen pages of text are distilled from fifteen pages of references. Although printing and lay-out are excellent the few photographs are poor.

This book fills a useful place in the libraries of hospitals that take few dermatology journals and of dermatologists who cannot keep up with the general medical literature as it affects skins and who are waiting for the next edition of their favourite dermatology text book.

C.F.Allenby

*British Journal of Dermatology* (1980) **102,** 370.

# *News and Notices*

## XVI International Congress of Dermatology

The XVI International Congress of Dermatology will be held in Tokyo, Japan, 23–28 May, 1982. The Congress includes a scientific programme (special lectures, case presentations, advances in dermatology, symposia, courses, workshops, informal discussion groups, free communications, poster communications, Japanese Dermatological Association seminars, and a scientific exhibition) and social events (performance of traditional Japanese Kabuki drama, a concert with a world-famous conductor, a short suburban sightseeing tour, and programmes for accompanying persons). The Congress site is the Hotel New Otani, Tokyo's prestige hotel which has been the site of many international congresses. English, French, Spanish, German and Japanese may be used in the Congress, and simultaneous interpretation will be provided during the main educational sessions.

The First Circular including detailed information regarding registration, hotel accommodation and group travel is now available on request to: Prof. Makoto Seiji, Secretary General, The XVI International Congress of Dermatology, C.P.O. Box 1560, Tokyo 100-91, Japan.

All interested persons are cordially invited to participate in the Congress.

## Fifth Annual Meeting of the American Dermatologic Society for Allergy and Immunology

A 3-day meeting devoted to allergic and immunological aspects of skin diseases will be held at the Royal Orleans Hotel in New Orleans, Louisiana on Thursday, Friday and Saturday, 25–27 September, 1980. Sessions will be devoted to Autoimmune Skin Diseases, Bullous Diseases, T-Cell Diseases, Dermatitis and Treatment. Tuition is £125. Twelve CME credit hours are offered. The ADSAI will meet jointly with the Louisiana Dermatological Society on Monday, 28 September. For information, contact Dr R.S.Rogers, III, Department of Dermatology, Mayo Clinic, Rochester, Minnesota 55901, U.S.A.

## New Trends in Allergy

An international symposium on New Trends in Allergy will be held in Munich, 1–3 August 1980, organized by the Dermatology Department of the Ludwig-Maximilians-University Munich in cooperation with the 'Deutsche Gesellschaft für Allergie- und Immunitätsforschung' and the 'European Society for Dermatological Research'.

The preliminary programme includes mast cells, immediate type mediators, delayed type hypersensitivity, allergy diagnosis, photoallergy, drug allergy, urticaria, atopic dermatitis, allergic vasculitis, contact dermatitis, advances in hyposensitization, insect sting allergy and side-effects of hyposensitization.

The organizing committee is: O.Braun-Falco, G.Burg, J.Ring, Dermatologische Universitatsklinik LMU, Frauenlobstrasse 11, D-8000 Munich 2, West Germany, from whom further information may be obtained.

# NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$ g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$) | μg | cubic millimetre(s) | mm$^3$ | micrometer(s) | μm |
| nanogram(s) ($10^{-}$ g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$ g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hour(s) | h | milliequivalent | mmol | | |

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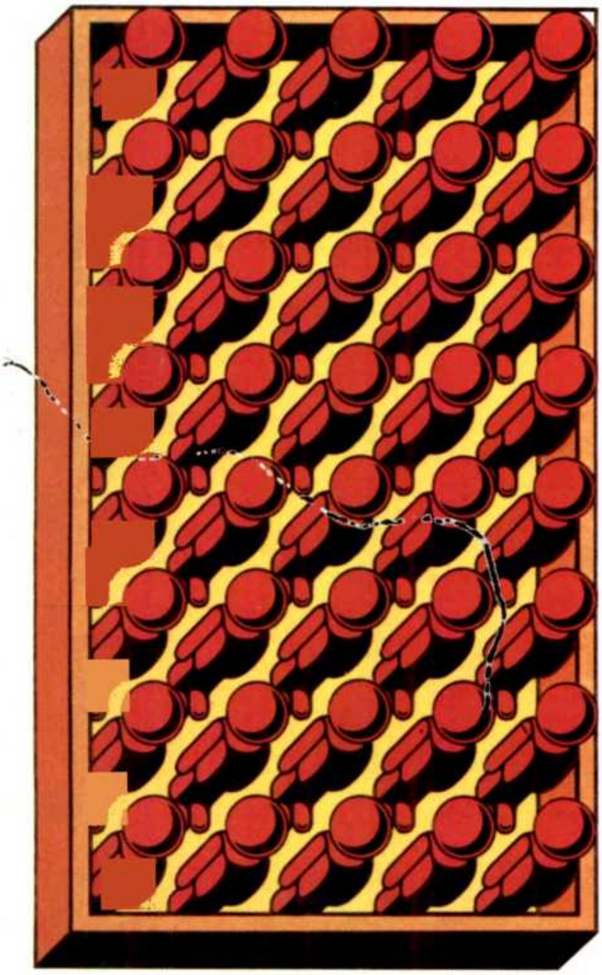

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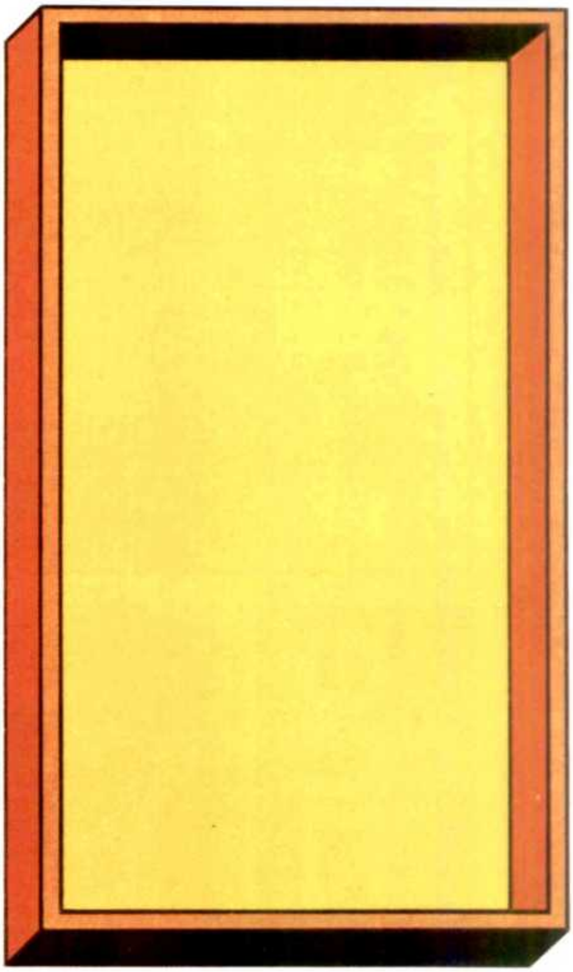

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Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

**Indications**

For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

**Directions for use**

Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

**Contraindications**

Acute psoriasis; dithranol sensitivity.

**Precautions**

Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

**Basic N.H.S. prices:**

Dithrocream 0.1% 50gm tubes £1.81
Dithrocream 0.25% 50gm tubes £1.96
Dithrocream Forte 50gm tubes £2.19

**Product Licence Nos.**

| | |
|---|---|
| Dithrocream 0.1% | PL 0173/0029 |
| Dithrocream 0.25% | PL 0173/0028 |
| Dithrocream Forte (0.5%) | PL 0173/0027 |

**References:**

1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
Dermal Laboratories Limited,
Gosmore, Hitchin, Hertfordshire SG4 7QR.

Dithrocream is a registered trade mark.

**Now available in three strengths**

# PUVA for Psoriasis

PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position. There are also Waldmann PUVA 180, 200 and 500 units for the treatment of hands, feet and other small areas.

A regime has been developed based on a London Teaching Hospital's experience and practice. The staff are professionally qualified and trained in the Photochemotherapy of psoriasis. Visits by Dermatologists are welcome by appointment.

An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

The Physician-in-Charge,
Psoriasis UVA (Medical Referals Only) Ltd.,
20 Harley Street, LONDON W.1.
Telephone 01-580 0875

flamazine™
SILVER SULPHADIAZINE 1% w/w cream

**flamazine cream**
-for infected leg ulcers and pressure sores

Flamazine presents the broad spectrum antibacterial action of silver in a non sensitising and soothing cream.

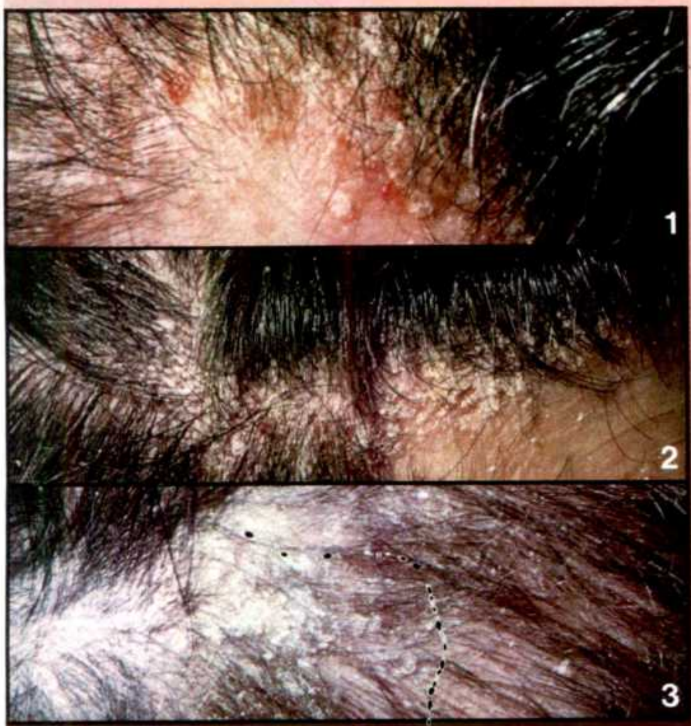
Further information is available on request from:

S&N SMITH & NEPHEW
Pharmaceuticals Ltd

BESSEMER ROAD, WELWYN GARDEN CITY, HERTFORDSHIRE AL7 1HF.
Telephone: WELWYN GARDEN (070 73) 25151

# Common Disorders of the Scalp.

They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

# Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

**Prescribing Information.**

**Presentation** Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

| | |
|---|---|
| Tar B.P. | 0.3% |
| Cade Oil B.P.C. | 0.3% |
| Coal Tar B.P.C. | 0.1% |
| Arachis Oil extract of crude coal tar | 0.3% |
| Oleyl alcohol | 1.00% |

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

**Uses** Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

**Dosage and Administration** The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

**Contra-indications, warnings, etc.** Nil

**Pharmaceutical Precautions** Store in a cool place

**Packs and Prices**

| | Pack | Basic NHS price |
|---|---|---|
| Polytar Liquid | 65ml | 57p |
| | 150ml | 82p |
| | 350ml | £1.48 |
| | 1 litre | £3.90 |
| Polytar Plus | 150ml | 90p |

**Product Licence Number**
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

**Stiefel Laboratories (UK) Limited**
Wellcroft Road, Slough SL1 4AQ

Simon was the unhappiest boy in his class

The psychological effects of eczema can be worse than the physical appearance justifies. Especially where children are concerned.

The patient with atopic eczema is often subjected to the ridicule and taunts of his classmates. And, all too soon, what should be the best days of his life can become the worst.

Treating him quickly and effectively is your objective.

But how?

You could opt for potent fluorinated steroids. They're certainly effective but concern has been expressed regarding the risk of side effects, particularly in children.

Hydrocortisone, although it's safer, isn't as effective.

Fortunately, there's Locoid.

Experimental studies[1,2,3] have shown that Locoid has a low capacity for side effects. But in terms of effectiveness, Locoid is as strong as the potent fluorinated steroids[4,5,6,7]

More important, it's as fast-acting[8,9]

Really, it's not so surprising that more and more Dermatologists and GP's are switching to Locoid.

Locoid

Hydrocortisone 17-butyrate 0.1%

Rely on its strength, trust in its

Full information available on request:
Brocades (Great Britain) Ltd. West Byfleet. Weybridge. Surrey KT14 6RA Tel: Byfleet 45536/42291

*Locoid is a trade mark for hydrocortisone 17-butyrate. P/L numbers: 0166/0058; 0166/0059; 0166/0060. C/I: In the presence of infection unless used with suitable chemotherapy. Large amounts and long duration in pregnancy and long-term therapy in infants. Apply two to four times daily. 30g cream/ointment £1.43, 20ml lotion £1.53. References: 1. Brit. J. Derm. (1976) 95,566. 2. Brit. J. Derm. (1977) 97,505. 3. Brit. J. Derm. Suppl. 12, Vol. 94 (1976). 4. Brit. J. Clin. Pract. (1973) 27,177. 5. Dermatologica (1976) 152 Suppl. 209. 6. Brit. J. Clin. Prac. (1972) 26,263. 7. Brit. J. Derm. (1970) 83,93. 8. Dermatologica (1976) 152 Suppl. 221. 9. Brit. J. Derm. (1973) 88,83*

# Difficult Dermatoses
# Dermovate

**(clobetasol propionate)**

Even long-standing, stubborn eczematous lesions respond quickly to Dermovate – with rapid relief from itching; and this is often followed by prolonged periods of remission. And because Dermovate is so rapidly effective, treatment need be of short duration only, minimising the risk of steroid side effects.

**Prescribing information**

**Dosage and administration**
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

**Side effects**
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.
This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing.
Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

**Precautions**
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.
Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

**Contra-indications**
Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines. Viral diseases of the skin, e.g., herpes simplex.

**Basic NHS cost (exclusive of VAT)**
Dermovate Cream or Ointment 25 gram tubes £1·74 (also availab in 100 gram tubes).

| Presentation | Product Licence numl |
|---|---|
| Dermovate Cream | 4/0219 |
| Ointment | 4/0220 |

***Glaxo***
**Leaders in topical steroid therapy**

Further information on Dermovate (trade mark) is available from:
Glaxo Laboratories Ltd
Greenford, Middlesex UB6 0HE

# People with psoriasis tend to hide themselves

It's not just the unsightly lesions. It's also the sheer discouragement, even despair. The available treatments seem to be either inadequate or cosmetically repugnant.

Alphosyl HC gives the psoriatic – and her doctor – grounds for hope. It is a cosmetically acceptable cream containing coal tar to slow down the psoriatic process, hydrocortisone in low dosage for a controlled anti-inflammatory effect, and allantoin to soften psoriatic plaques and aid in their removal.

A published clinical study* has shown Alphosyl HC to be broadly comparable in efficacy to betamethasone, and somewhat superior in its descaling action. And Alphosyl HC is preferable on safety grounds since it does not contain a fluorinated steroid.

## Alphosyl HC

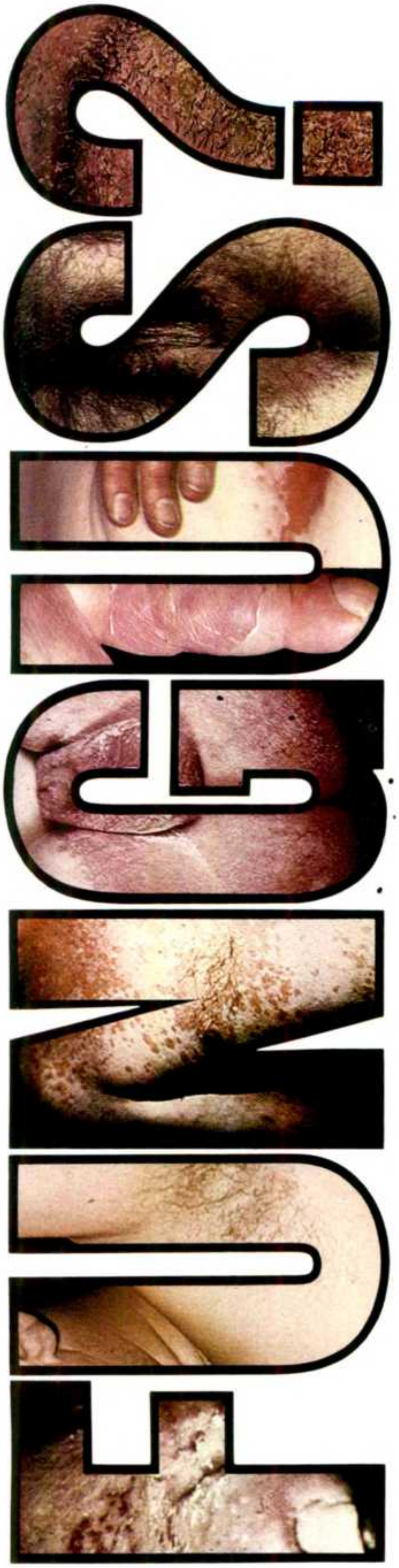
**hydrocortisone 0.5% with allantoin and coal tar extract.**

**purpose-designed for psoriasis**

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol. 33 No. 4, 106.

**Presentation** A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, u.s.p. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and Administration** Apply two to four times daily massaging vigorously into the affected areas. **Contra-indications and Warnings, etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tubes of 30gm and 45gm. **Basic NHS cost** £1.62 for 30gm, £2.29 for 45gm. **Product Licence No.** 0036/0026

Further information is available from
**Stafford-Miller Limited**, Professional Relations Division, Hatfield, Herts. AL10 9NZ



**Canesten**®
clotrimazole

**Effective against all fungal skin infections**

So effective treatment with Canesten may begin at once – without waiting for culture results.

Wherever on the body a fungal infection occurs, whatever the organism responsible, there is a need for speedy effective therapy. Canesten's broad range of presentations is the answer… cream, atomiser spray and now Canesten powder for adjunctive therapy and to help prevent re-infection.

**Canesten**
**the most widely-prescribed antifungal**

**Prescribing Information**

**Presentations** *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder containing 1.0% clotrimazole.

**Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections.

**Dosage and Administration** *Cream, Atomiser Spray* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection.

**Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation.

**Precautions** Canesten atomiser spray should not be used near a naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled.

**Product Licence Numbers** Cream: PL 0010/0016. Atomiser Spray: PL 0010/0060. Powder: PL 0010/0067.

**Basic NHS prices** Cream: 20g, £1.37; 50g, £3.20. Atomiser Spray: 40ml, £3.68. Powder: 30g, £0.99.

Further information is available from
Bayer UK Limited, Pharmaceutical Division,
Haywards Heath, West Sussex RH16 1TP.
Telephone Haywards Heath (0444) 57911.

Distributed in the Republic of Ireland by ·
Bayer (Ireland) Limited, Dun Laoghaire, County Dublin.

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**sentation** Sterile, straw-coloured spherical beads of dextranomer of
0.3 mm diameter, packed in plastic castors or single-use sachets.
**es** A dressing for moist, small area burns, wounds and indolent ulcers, whether
an or infected.
**sage and administration** A 3 mm layer of Debrisan should be sprinkled on
he wound and kept in place by a pad of lint or a perforated plastic sheet.
risan is hydrophilic and the tissue exudate is drawn up into the layer. The
risan should be renewed before saturation occurs. Depending on the rate of
dation this may be necessary from one to five times a day. Twice daily is usually adequate. When Debrisan is changed the old material is readily rinsed off with water or saline and new material may then be sprinkled on to the wound. Full instructions for use are enclosed with each pack. Treated in this manner the wound will remain soft and pliable during healing.

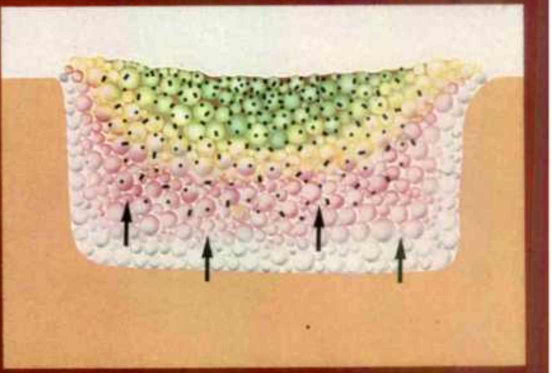
**Contra-indications, warnings, etc.**

1. Do not leave Debrisan for more than 24 hours on wounds with a very low exudation rate as it may dry and form a crust which may be difficult to wash off.
2. Occlusive dressings may lead to maceration of skin round the wound under treatment.

**Where** On all secreting wounds such as leg ulcers, pressure sores, post-surgical infections, Debrisan actively speeds healing.

**When** As soon as such wounds present, apply Debrisan and renew as necessary. 'The quality as well as the rapidity of wound healing has been impressive.'[1]

**How** By capillary action Debrisan continuously removes wound exudate, with bacteria and fibrinogen drawn up into the top layer. Inflammatory mediators are removed and painful oedema then subsides. Local blood flow should improve and the wound base is rendered clean and granulating.



# Debrisan

dextranomer

## ℞ DEBRISAN

Unique fast-acting wound therapy

---

[1] Thorne N. (1979) Brit. J. clin. Pract. **33**, 21

3. When deep infected wounds are treated, care must be taken to wash Debrisan from the depths of the wound.
4. No side-effects have been reported.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Precautions:

1. Not to be used on dry wounds.
2. When exudate has been markedly removed by Debrisan alternative treatment should be substituted.
3. In order to avoid cross-infection it is recommended that the contents of a castor be confined to the treatment of a single patient for one day.

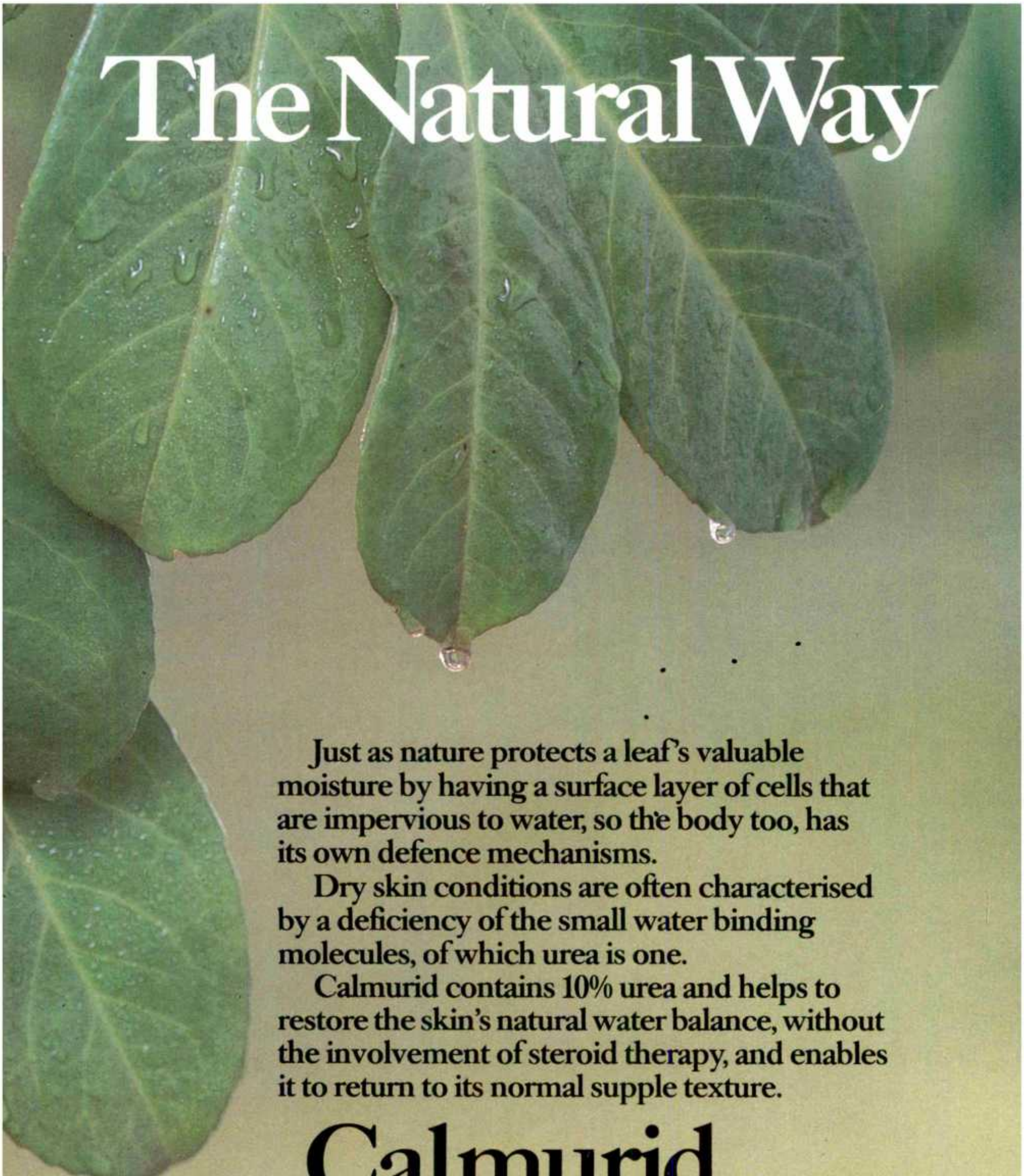
**Pharmaceutical precautions** Keep in a dry place in well-closed containers.

**Package quantities** Sachets of 4 g in boxes of seven. Basic NHS price £1.60 each. Castors of 60 g.

**Legal category** POM. **PL** 0009/0021

Debrisan (regd) dextranomer is a product of Pharmacia (Great Britain) Ltd, Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321

Further information is available on request to the Company.



Just as nature protects a leaf's valuable moisture by having a surface layer of cells that are impervious to water, so the body too, has its own defence mechanisms.

Dry skin conditions are often characterised by a deficiency of the small water binding molecules, of which urea is one.

Calmurid contains 10% urea and helps to restore the skin's natural water balance, without the involvement of steroid therapy, and enables it to return to its normal supple texture.

**Calmurid**

urea

**helps the skin regain its moisture**

**Prescribing Information**

**Presentation.** A white shiny cream containing Carbamide (urea) B.P. 10% in a stabilising emulsified base.

**Uses.** Treatment of dry hyperkeratotic skin conditions.

**Dosage and Administration.** After washing the affected skin apply a thick layer and leave 3-5 minutes. Apply twice daily, reducing dosage with improvement. Hyperkeratotic feet: soak in warm water dry with rough towel and apply as above.

**Contra-indications, warnings etc.** Smarting may occur on fissured or sensitive skin.

**Pharmaceutical Precautions.** Store in a cool place.

**Legal Category.** G.S.L.

**Package Quantities.** Tubes of 50g, 100g and 300g.

**Further Information.** Lanolin, preservative and steroid free. PL 0009/5009.

50g Calmurid Cream £1.25 Basic N.H.S. price

**Pharmacia**

Calmurid (regd.) urea B.P. 10% cream is a product of Pharmacia (Great Britain) Ltd., Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321 Further information is available on request to the Company.

# When efficacy is the need and safety the concern

No need now to sacrifice efficacy for safety because Eumovate fulfils the need for a corticosteroid preparation with greater topical activity than hydrocortisone, yet with a wide margin of safety.

# Eumovate

**(clobetasone butyrate)**

**a unique balance of efficacy and safety**

**Prescribing information**

**Uses**

Eumovate is suitable for treating the
forms of eczema, seborrhoeic derma
and other steroid responsive skin
conditions.

**Dosage and administration**

Apply up to four times a day until
improvement occurs, when the frequ
of application may be reduced.

**Contra-indications**

Bacterial, fungal or viral diseases of t
skin.

**Precautions**

In infants and children, long-term
continuous topical corticosteroid the
should be avoided where possible, as
adrenal suppression can occur. In inf
the napkin may act as an occlusive
dressing, and increase absorption. Th
least potent corticosteroid which will
control the disease should be selected
Appropriate antimicrobial therapy sho
be used concurrently whenever treati
inflammatory lesions which have bec
infected.

Any spread of infection requires
withdrawal of topical corticosteroid
therapy and, possibly, commenceme
systemic chemotherapy.

As with all corticosteroids, prolonged
application to the face is undesirable.

Topical steroids should not be used
extensively in pregnancy, i.e., in large
amounts or for prolonged periods.

**Side effects**

In the unlikely event of signs of
hypersensitivity appearing, applicatio
should stop immediately. If large area
were to be treated, some patients cou
absorb sufficient steroid to cause tran
adrenal depression despite the low de
of systemic activity associated with
clobetasone butyrate.

Local atrophic changes could occur w
moisture increases absorption of stero
but only after prolonged use.

**Basic NHS cost** (exclusive of VAT)
25 gram tube £1·48 (also available in
100 gram tubes).

**Product Licence numbers**

Cream: 4/0233
Ointment: 4/0254

## Glaxo

**leaders in topical steroid the**

Further information on Eumovate (tra
mark) is available from:
Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

# New books from Blackwell

## Investigative Techniques in Dermatology

Edited by R. Marks M.B. B.Sc. F.R.C.P. 1979. 288 pages, 102 illustrations. £14.50

This book provides a practical link between the purely theoretical works on the skin and its diseases and laboratory manuals. It particularly emphasises the advantages and disadvantages of commonly used techniques and how they can most effectively be employed.

Contents: Looking at skin lipids; Technique of patch tests; Immune complexes; The investigation of fibrinolytic activity; Methods for microbiological investigations for acne; The investigation of photodermatoses; Identification of mediators of inflammation; Genetic techniques in dermatology; Transmission electron microscopy; *In vitro* tests of cell mediated immunity; Epidermal cell kinetics; The application of scanning electron microscopy to dermatological problems; Clinical trials in dermatology; Techniques for the examination of connective tissue

## International Coding Index for Dermatology

Compiled by Suzanne Alexander and Alan B. Shrank. 1978. 112 pages. Paper, £2.00

This new index is designed to be compatible with the WHO International Classification of Disease for the accurate and efficient retrieval of information on dermatological diagnoses. It uses codes which are understood and accepted universally for epidemiological studies, for the planning of health services and for medical research. It is compact and inclusive, and provides an invaluable reference for every practising dermatologist

## Textbook of Dermatology

Edited by A. Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. Third Edition, 1979. 2500 pages, 1131 illustrations. Two volumes, £87.50

A new edition of a work of this stature is a major event which will be widely welcomed, especially by the many thousands of dermatologists throughout the world who have already ordered it in advance of publication. Significant advances in our knowledge of the skin and its diseases which have occurred during the last seven years have been incorporated into the text while some of the older material has been removed in order to keep the volumes within a manageable size. Several chapters have been revised by new members of the team of contributors and their fresh approach has greatly enhanced the presentation of the subjects under their charge. As in its two predecessors, the editors have designed this new edition to provide a textbook which is soundly based in science, but which is nevertheless essentially a practical work of reference for the serious student of dermatology, whose main responsibility is the clinical care of his patients.

## Essentials of Skin Disease Management

D.J. Barker M.B. M.R.C.P. and L.G. Millard M.B. M.R.C.P. 1979. 96 pages, 25 illustrations. Paper, £3.25

This is a practical little book on the principles behind the use of pastes, creams and other therapeutic aids. It is designed to provide quick and easy reference for a wide readership including students, nurses, general practitioners and pharmacists

Contents: Topical therapy; Systemic therapy; Dressing technique; Management of common skin disorders; Formulary

**Blackwell Scientific Publications**

# 'The most active fungicide available'

Tinea capitis

- **Ecostatin is the most effective treatment against a wide range of yeasts, moulds and dermatophytes (1)**
- **Ecostatin topicals are presented in a form to suit all body areas**
- **Ecostatin is cosmetically acceptable, non staining and well tolerated**
- **Ecostatin is also available as pessaries for vaginal infections and a twin-pack containing pessaries and cream for treating the anogenital region and the male consort**

**Tinea corporis/capitis** 90% of patients cured clinically and mycologically in 4.3 weeks (2)

**Pityriasis versicolor** 100% patients cured clinically and microscopically in 1-2 weeks (3)

**Tinea manuum** 69% patients cured clinically and mycologically in 4.8 weeks (2)

**Tinea cruris** 76% patients cured clinically and mycologically in 6 weeks (4)

**Tinea pedis** 89% patients cured clinically and mycologically in 4.8 weeks (2)

# Ecostatin™

**econazole nitrate**

**ECOSTATIN***
Pessaries: Each contain 150 mg econazole nitrate. Twin Pack: Three pessaries plus 15 g Ecostatin cream. Cream, Lotion, Powder, Spray Powder and Spray Solution: All contain 1% w/w econazole nitrate.

**Indications**
Pessaries and Twin Pack: Vulvovaginal candidosis. Cream, Lotion, Powder, Spray Powder and Spray Solution: All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi.

**Actions**
Econazole nitrate is a broad-spectrum antifungal agent.

**Dosage and Administration**
Pessaries and Twin Pack: One pessary to be inserted at bedtime for three consecutive nights. The cream to be applied twice daily to the anogenital area. Cream, Lotion, Powder, Spray Powder and Spray Solution: To be applied twice daily to the affected area.

**Precautions**
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing. Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis. The Spray Solution should not be used on mucous membranes.

**Side-effects**
Occasional local irritation. The pessaries may cause transitory discomfort.

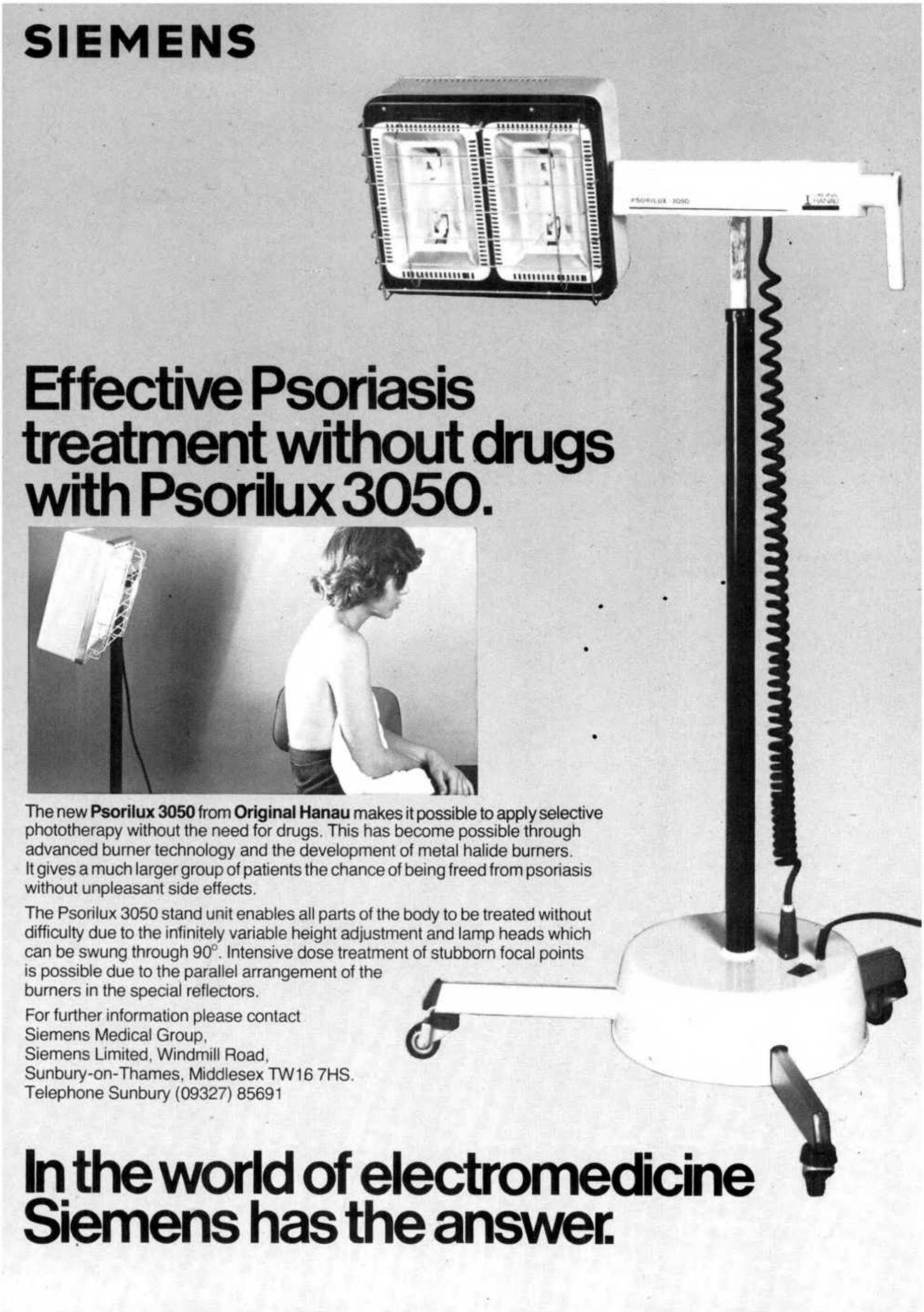
**Package quantities, prices and Product Licence Numbers**
**Pessaries:** Pack of 3 with applicator – £3.60
PL No. 0085/0013
**Twin Pack:** 3 pessaries, applicator and 15 g cream – £4.70
**Cream:** 15 g – £1.25; 30 g – £2.50
PL No. 0085/0014
**Lotion:** 30 ml – £2.50
PL No. 0085/0021
**Powder:** 30 g – £2.90
PL No. 0085/0023
**Spray Powder:** 200 g – £2.40
PL No. 0085/0020
**Spray Solution:** 150 g – £3.00
PL No. 0085/0022

**References:**
**(1) Grupper, Ch. and Avram, A. Proceedings of VIth ISHAM Congress, Tokyo, June 1975, p. 162**
**(2) Schwartz, K. J. et al Deutsch. med. Wochenschr., 1975,** *100,* **1497.**
**(3) Gisslen, H. et al Curr. ther. Res., 1977,** *21,* **681**
**(4) Dorn, M. et al Munch. med. Wschr. 1975** *117,* **687**

**Product Licence Holder**
F.A.I.R. Laboratories Ltd.,
179 Heath Road,
Twickenham, TW1 8BJ

Further information is available from
**FAIR Laboratories Ltd., 179 Heath Road, Twickenham, Middx. TW1 8BJ**

** Ecostatin is a registered trademark and FAIR Laboratories is the authorised user*



**SIEMENS**

# Effective Psoriasis treatment without drugs with Psorilux 3050.

The new **Psorilux 3050** from **Original Hanau** makes it possible to apply selective phototherapy without the need for drugs. This has become possible through advanced burner technology and the development of metal halide burners. It gives a much larger group of patients the chance of being freed from psoriasis without unpleasant side effects.

The Psorilux 3050 stand unit enables all parts of the body to be treated without difficulty due to the infinitely variable height adjustment and lamp heads which can be swung through 90°. Intensive dose treatment of stubborn focal points is possible due to the parallel arrangement of the burners in the special reflectors.

For further information please contact
Siemens Medical Group,
Siemens Limited, Windmill Road,
Sunbury-on-Thames, Middlesex TW16 7HS.
Telephone Sunbury (09327) 85691

**In the world of electromedicine Siemens has the answer.**

# RoC. A laboratory with over 20 years experience in the research and development of hypo-allergenic formulae.

In the field of hypo-allergenic cosmetology RoC enjoys a high reputation amongst French dermatologists. For over 20 years now the company has concentrated its efforts on the creation of skin care, treatment and make-up preparations formulated for highly sensitive and allergic skins. All common allergens have been eliminated from RoC formulae which are characterised by their purity and simplicity.

Perfumes, colourants and preservatives, of a photosensitising or irritant nature, known to cause redness, dermatitis or eczema have been systematically excluded.

Creams are always presented in sealed metal tubes and all the products are guaranteed by a date of freshness.

RoC preparations are manufactured in the same way as pharmaceutical products in that they are controlled before, during and after production, and finally tested in a hospital and clinical environment, to offer the best guarantee of safety for the most sensitive of skins.

RoC

**HYPO-ALLERGENIC PRODUCTS GUARANTEED WITHOUT PERFUME.**
**MADE IN FRANCE**

RoC hypo-allergenic products are sold in a certain number of selected chemists (and in the pharmacy department of some department stores).

For further information contact Mary Wray, telephone: 01-493 7785.
Laboratoires RoC (UK) Ltd., 46 Mount Street, London W1Y 6EJ or write directly to:
The Medical Director, Laboratoires RoC,
50, rue de Seine, 92700 Colombes - France.

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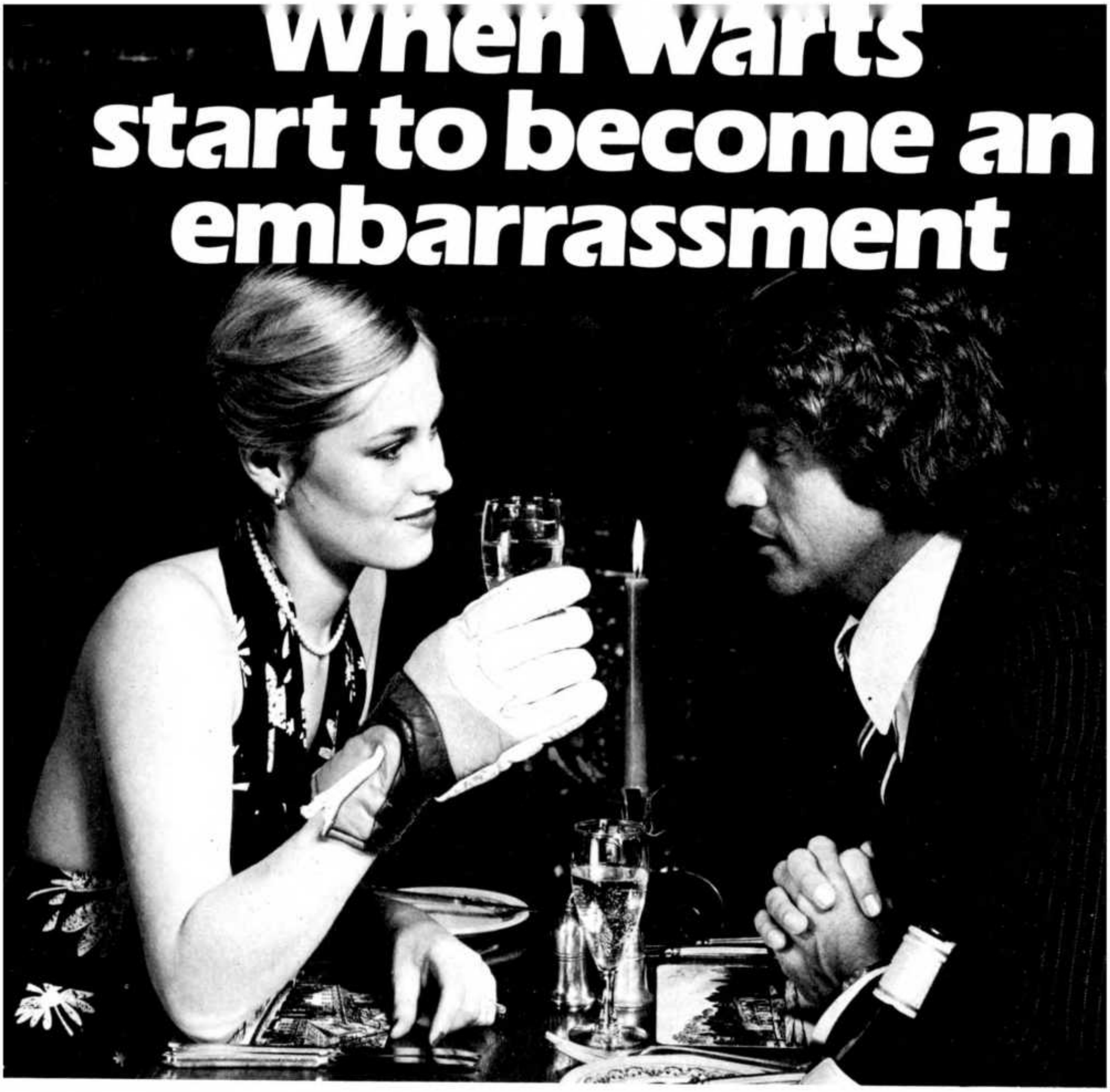

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*British Journal of Dermatology* (1980) **102**, 371.

*Clinical and Laboratory Investigations*

# A comparison of IgA positive and IgA negative dapsone responsive dermatoses

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## SUMMARY

A study of thirty-three patients with a clinical diagnosis of dermatitis herpetiformis (DH) referred to our DH clinic over the last 11 years is reported. Twenty-six were referred by other consultant dermatologists. The diagnosis had been made by the clinical features and response of the rash to dapsone. Seventeen patients were found to have IgA in the uninvolved skin (IgA positive) and in sixteen no IgA was found (IgA negative). The duration of the rash prior to referral to the DH clinic was 3 months to 19 years (mean 5·0 years) for the IgA negative patients and 2 months to 22 years (mean 5·2 years) for the IgA positive group. The length of follow-up was 3 months to 11 years (mean 5·0 years) for the IgA negative, and 2–11 years (mean 5·6 years) for the IgA positive group. During follow-up the rash cleared completely and required no treatment in seven of the sixteen IgA negative patients. Thirteen of these sixteen patients no longer required dapsone, but six patients were receiving alternative treatment. In the three patients still taking dapsone IgA has not been found on subsequent biopsy. Of the seventeen IgA positive patients only three were able to stop dapsone during follow-up and in these three the IgA was still detected in the skin. Small intestinal mucosa was abnormal in eight of eleven IgA positive patients, but was normal in all thirteen IgA negative patients in whom jejunal biopsies were performed.

An alternative diagnosis to DH has subsequently been made in thirteen of the sixteen IgA negative patients. Although the significance of IgA in the skin in DH is not known it appears to be part of the disease process. Patients who have a rash suggestive of DH and which is dapsone responsive, but in whom IgA is not found in the uninvolved skin, usually turn out to have a dermatosis other than dermatitis herpetiformis.

Referral to a unit with expertise in immunofluorescence techniques of skin biopsies would appear to be helpful.

IgA deposits in the uninvolved skin in dermatitis herpetiformis (DH) were first described by van der Meer (1969). In a review of the diagnostic criteria for DH, Fry & Seah (1974) stated that the presence of IgA in the uninvolved skin was the best single criterion and that the diagnosis should not be made

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unless IgA is found. However, this view has been disputed by other workers (Marks, 1974; Alexander, 1975a; Marks, 1977, 1978; Cooper & Cooke, 1978; Ross *et al.*, 1978). The other diagnostic criteria most commonly employed are the clinical and histological features and response of the rash to dapsone. We thought it would be of interest to report our follow-up study of patients referred to our DH clinic over the last 12 years, with a dapsone responsive dermatosis, and compare those in whom IgA was not found in the uninvolved skin with a similar group in whom IgA was found.

## PATIENTS AND METHODS

During the last 12 years, sixteen patients were seen in a special clinic for patients with DH who had a rash which was clinically suggestive of DH and responded to dapsone but who were IgA negative. Eleven of the sixteen patients were referred to the clinic by other consultant dermatologists. The five other patients were referred by the staff of St Mary's Hospital Skin Department. These sixteen IgA negative patients have been compared to seventeen patients who also had a rash suggestive of DH which responded to dapsone but who were IgA positive. Of the seventeen IgA positive patients thirteen were referred by other consultant dermatologists, and four by the staff of St Mary's Hospital. Patients were referred to our clinic for a variety of reasons: (i) investigation of small intestine; (ii) treatment with a gluten-free diet; (iii) confirmation of diagnosis; (iv) because of our known interest in this disease.

The age range in the IgA negative patients was 11–60 years with a mean of 38·3, and 18–65 in the IgA positive group with a mean of 32·9. There were eight males and eight females in the IgA negative group and eleven females and six males in the IgA positive patients.

### *Dapsone requirements*

Patients were instructed to take the smallest possible dose of dapsone to control their eruption throughout the study.

During the period of follow-up patients were given other treatment (e.g. topical corticosteroids for eczema) if an alternative diagnosis was suspected.

### *Skin biopsies for histology*

These were performed in ten of the sixteen patients who were IgA negative and in eight of the seventeen patients who were IgA positive. The results are expressed as the conclusion of the histopathologist.

### *Small intestinal biopsies*

These were performed successfully in thirteen of the sixteen IgA negative patients and in eleven of the seventeen IgA positive patients. Both the macroscopic appearance of the intestinal mucosa and the intra-epithelial lymphocyte count per 1000 epithelial cells were assessed as described by Fry *et al.* (1972). A count below 300 has previously been taken as normal.

### *Diet*

Nine of the IgA positive and two of the IgA negative patients had tried a gluten-free diet (GFD) for a short period (approximately 3 months) but were unable to adhere to the diet, thus were asked to discontinue it. Otherwise all patients were taking a normal diet.

### *IgA in skin*

This was detected by direct immunofluorescence as described by Seah *et al.* (1972). The biopsies were taken from clinically uninvolved skin. The biopsies were repeated in the three patients who were originally IgA positive and were able to stop dapsone, and in the three IgA negative patients who still required dapsone.

## RESULTS

### *Duration of rash and follow-up (Tables 1 and 2)*

The duration of the rash prior to being seen in the DH clinic was similar in both groups. In the IgA negative group it varied from 3 months to 19 years, with a mean of 5·0 years and in the IgA positive group it was 2 months to 22 years with a mean of 5·2 years.

The period of follow-up has ranged from 3 months to 11 years with a mean of 5·0 years in the IgA negative group and from 2 to 11 years with a mean of 5·6 years in the IgA positive group.

During the period of follow-up the rash has cleared completely in seven of the sixteen (44%) IgA negative patients, and in three of the seventeen (18%) IgA positive patients, and none of these patients is receiving any treatment at the present time. The total duration of the rash before clearing ranged from 8 months to 20 years with a mean of 8·2 years in the seven IgA negative patients, and from 3 to 9 years with a mean of 5·7 years in the three IgA positive patients.

### *Distribution of rash (Table 3)*

There was a higher incidence of involvement of the elbows in the IgA positive compared to the IgA negative group, the latter having a higher incidence of involvement elsewhere on the arms. There was a slightly higher incidence in the involvement of the buttocks in the IgA positive patients but the incidence for the shoulders was the same.

### *Presence of blisters*

Blisters were seen in seven of the sixteen IgA negative patients, but in sixteen of the seventeen IgA positive patients.

### *Dapsone requirements*

The dose of dapsone required to control the eruption, when first seen in the DH clinic, was 50–300 mg per day (mean 148 mg) in the IgA negative group, and 50–300 mg per day (mean 112 mg) in the IgA positive group.

### *Dapsone requirements on follow-up*

Fourteen of the seventeen (82%) IgA positive patients still require dapsone. The three patients who have been able to stop dapsone without recurrence of their eruption are not receiving any other treatment. Only three of the sixteen (19%) IgA negative patients still require dapsone. One patient (No. 6) has subcorneal pustular dermatosis, one patient (No. 12) has been diagnosed as eczema but still finds dapsone helpful in controlling his eruption, and one patient (No. 14) in whom no diagnosis has been made takes dapsone intermittently, and no other treatment is of help. This patient's dapsone requirements have fallen over the 8 year follow-up from 100 mg daily to 100 mg daily for periods of 1–2 weeks, two to three times per year when the rash appears. The patient with pemphigus (No. 11) has been able to stop treatment after 16 years. Originally she received dapsone, which controlled the eruption, but side-effects developed, and sulphapyridine was given which also controlled the rash.

### *Skin biopsies for histology*

Of the nine biopsies from the IgA negative patients only two were reported as DH. Pemphigus was

TABLE 1. IgA negative patients

| Patient No. | Sex | Age when first seen in DH clinic (years) | Duration of symptoms before attendance in DH clinic (years) | Duration of follow-up in DH clinic (years) | Duration of rash before remission (years) | Dose of dapsone (mg/day) | When alternative diagnosis made after first attendance in DH clinic (months) | Dapsone requirements when last seen (mg/day) | Skin biopsy | Gut biopsy* | Alternative treatment | Final diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 48 | 18 months | 11 | 12½ | 100 | 7 years | Nil | | F&L 173 | Topical steroid | Eczema |
| 2 | F | 36 | 4 | 5¼ | 7 | 100 | 6 | Nil | Non specific | F&L 285 | Topical steroid | Eczema |
| 3 | F | 37 | 19 | 5 | 20 | 100 | 6 | Nil | | F&L 138 | Topical steroid | Papular urticaria |
| 4 | M | 49 | 10 months | 4¼ | | 200–300 | 3 | Nil | Eczema | F&L 59 | Antihistamines | Eczema |
| 5 | M | 26 | 5 | 3 months | 5¼ | 100 | 3 | Nil | | F&L 180 | Topical steroid | Eczema |
| 6 | M | 25 | 1 | 1 | | 300 | 1 | 200 | Sub-corneal pustular dermatosis | F&L 174 | | Subcorneal pustular dermatosis |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | M | 24 | 3 months | 4½ | 2½ | 200 | | Nil | DH | F&L<br>116 | Topical<br>steroid | ? |
| 8 | M | 25 | 3 | 3½ | | 100 | 1 | Nil | Non<br>specific | F&L<br>179 | Topical<br>steroid<br>antihistamines | Eczema |
| 9 | M | 54 | 3 | 5½ | | 150 | 18 | Nil | Non<br>specific | F&L<br>357 | Topical<br>steroid<br>Antihistamines | Eczema |
| 10 | F | 42 | 10 | 18 months | | 200 | 1 | Nil | | F&L<br>355 | Topical<br>steroid<br>Antihistamines | Eczema |
| 11 | F | 43 | 11 | 6½ | | 200 | 1 year | Nil | Pemphigus | F&L<br>253 | Sulphapyradine | Pemphigus |
| 12 | M | 60 | 4 | 6½ | | 100 | 6 | 100 | Non<br>specific | F&L<br>148 | Topical<br>steroid | Eczema |
| 13 | M | 65 | 6 months | 5 | 8 months | 300 | 2 | Nil | DH | L<br>91 | Prednisone<br>Antihistamines | Eczema |
| 14 | M | 56 | 8 | 8 | | 100 | | 100† | Eczema | | Topical<br>steroid | ? |
| 15 | F | 12 | 7 | 6 | | 50 | 18 | Nil | | | Topical<br>steroid | Eczema |
| 16 | F | 11 | 11 months | 5 | 18 months | 50 | | Nil | | | | ? |

*Gut biopsy F & L = Finger and leaves; number is the intra-epithelial lymphocyte count.
† Only takes dapsone 100 mg daily for 1–2 weeks, two or three times a year.

TABLE 2. IgA positive patients

| Patient No. | Sex | Age when first seen in DH clinic (years) | Duration of symptoms before attendance in DH clinic (years) | Duration of follow-up in DH clinic (years) | Duration of rash before remission (years) | Dose of dapsone (mg/day) | Dapsone require-ments when last seen (mg/day) | Skin biopsy | Gut biopsy* | Alternative treatment | Final diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | F | 58 | 5 | 4½ | | 100 | 100 | | F&L<br>295 | | DH |
| 18 | M | 20 | 1 | 7 | | 100 | 100 | | Convoluted<br>578 | | DH |
| 19 | M | 65 | 2 | 10 | | 100 | | Erythema multiforme | F&L<br>235 | Sulphamethoxy-pyridazine* | DH |
| 20 | F | 19 | 5 | 3½ | | | Nil | DH | | | DH |
| 21 | F | 44 | 6 | 3 | | 50 | 50 | | | | DH |
| 22 | M | 30 | 3 | 3½ | | 100 | 100 | | | | DH |
| 23 | M | 36 | 5 | 4 | 9 | 50 | Nil | | F&L<br>125 | | DH |
| 24 | F | 23 | 1 | 9 | | 100 | 50 | DH | F&L<br>445 | | DH |
| 25 | M | 48 | 4 months | 10 | | 100 | | Eczema | | Sulphamethoxy-pyridazine† | DH |
| 26 | F | 23 | 8 | 10 | | 100 | 200 | | Leaves<br>588 | | DH |
| 27 | M | 53 | 22 | 6 | | 100 | 100 | DH | Flat<br>568 | | DH |
| 28 | F | 34 | 10 | 9 | | 100 | 50 | DH | Convoluted<br>385 | | DH |
| 29 | F | 18 | 8 | 10 | | 100 | 100 | DH | Flat<br>313 | | DH |
| 30 | F | 25 | 2½ | 3 | 5½ | 150 | Nil | | | | DH |
| 31 | M | 23 | 6 | 2 | | 300 | 300 | | Convoluted<br>954 | | DH |
| 32 | F | 23 | 6 | 5 | 8 | 100 | Nil | DH | | | DH |
| 33 | F | 56 | 12 | 11 | | 100 | 50 | | Convoluted<br>477 | | DH |

*Gut biopsy F & L = Finger and leaves; number is the intra-epithelial lymphocyte count.
† Patient developed side-effects to dapsone, changed to sulphamethoxypyridazine.

TABLE 3. Distribution of rash

| | Total | Elbows | Knees | Buttocks | Upper back | Face | Abdomen | Arms | Legs | Chest | Scalp | Axilla |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IgA Positive | 17 | 12(71%) | 5(29%) | 9(53%) | 11(65%) | 5(29%) | 4(24%) | 2(12%) | 4(24%) | 2(12%) | 2(12%) | 2(12%) |
| IgA Negative | 16 | 4(25%) | 2(13%) | 5(31%) | 10(63%) | 1(6%) | 6(38%) | 8(50%) | 10(63%) | 2(13%) | 3(19%) | 0 |

TABLE 4. Final diagnosis in IgA negative patients

| Diagnosis | Number |
|---|---|
| Eczema | 10 |
| Papular urticaria | 1 |
| Pemphigus | 1 |
| Subcorneal pustular dermatosis | 1 |
| Undiagnosed | 3 |

diagnosed in one, subcorneal pustular dermatosis in one, eczema in two, and non-specific inflammatory changes in four. Of the eight biopsies carried out in the IgA positive group, six were suggestive of DH, one was reported as eczema and one as erythema multiforme.

### *Small intestinal biopsies*

Of the thirteen biopsies carried out in the IgA negative group all showed a normal macroscopic appearance. The intra-epithelial lymphocyte count was greater than 300 in only two of the thirteen biopsies and in both these the count was only slightly above 300, being 355 in one and 357 in the other. Of the eleven biopsies in the IgA positive group the macroscopic appearance was abnormal in six, and the lymphocyte count was above 300 in eight of the eleven biopsies.

### *Alternative diagnosis (Table 4)*

Since the initial referral to the DH Clinic, an alternative diagnosis was made on clinical and, in some, histological and immunological findings, in thirteen of the sixteen IgA negative patients. Eczema has been diagnosed in ten, papular urticaria in one, pemphigus in one, and subcorneal pustular dermatosis in one. No diagnosis has been made in the remaining three patients. The duration of follow-up in the DH clinic before an alternative diagnosis was made in the IgA negative patients ranged from 1 month to 7 years (mean 7 months). However, the total duration of the rash prior to alternative diagnosis ranged from 1 year to 19·5 years (mean 6·5 years). Once an alternative diagnosis was considered other appropriate treatment apart from dapsone was given.

### *Repeat skin biopsies for IgA*

IgA was still present in the three patients in the IgA positive group who no longer required dapsone. These patients had no rash and were not receiving any treatment. In the IgA negative group the test was repeated in the three patients who still take dapsone. In all these three patients no IgA was detected in the second biopsy.

## DISCUSSION

These results support the suggestion (Fry & Seah, 1974) that the presence of IgA in the uninvolved skin is the most reliable criterion in establishing a diagnosis of DH. This view has been supported by Chorzelski & Jablonska (1975), Seah & Fry (1975) and Katz & Strober (1978). Chorzelski & Jablonska (1975) stress the importance of taking serial sections of the biopsies if no IgA is found on the first sections, and Seah & Fry (1975) consider a second biopsy should be performed if no IgA is found in the first and the clinical picture strongly suggests DH. Katz & Strober (1978) reported two out of fifty-three patients in whom no IgA was found, and they considered that these two IgA negative patients may not have DH and that follow-up would probably lead to an alternative diagnosis. However, the presence of IgA in establishing the diagnosis of DH has been questioned by Marks (1977, 1978) Cooper & Cooke (1978) and Ross *et al.* (1978). Cooper & Cooke (1978) reported a series of twenty patients whom they claimed had DH but IgA was found in only ten. All their patients were taking a GFD, for periods ranging from 1 to 10 years, and although it is possible that the IgA had disappeared from the skin in response to a GFD (as reported by Harrington & Read, 1977) this seems unlikely as small intestinal abnormality was still present. In addition other workers have not found IgA to disappear from the skin in response to a GFD (Fry *et al.*, 1978; Katz & Strober, 1978; Reunala, 1978). Marks (1977, 1978) considered the IgA test for DH to be 'non-specific' and her evidence for this was based on her findings that two out of fifty-five patients considered to have DH by other criteria did not have IgA in the skin, and four out of 141 patients were found to have IgA in the skin, in a pattern and distribution similar to that found in DH, but in whom subsequently a different diagnosis was made. Although more than one biopsy was performed in the IgA negative group it is not stated whether serial sectioning was carried out. It is more difficult to account for the IgA in those patients considered not to have DH but it is not stated why these patients had biopsies from the uninvolved skin, for studies for immunoglobulin deposits. It is known that IgA may be found in other dermatological conditions, e.g. cicatricial pemphigoid and lupus erythematosus, but this is usually in the rarer continuous pattern, and not the papillary pattern characteristic of DH. In addition Blenkinsopp, Clayton & Haffenden (1978) have found IgA in one of their fifty-five specimens from control subjects. Ross *et al.* (1978) reported IgA in the skin of eight out of fifteen patients with coeliac disease. However, only four of the eight patients had the pattern and distribution of IgA as seen in DH, the others had patterns of deposition described as 'globules', which have been described in normal individuals (Seah *et al.*, 1972). It seems probable that these results in coeliac disease may be false positives and due to technical errors as other reports have not found IgA in the skin in coeliac disease (Seah *et al.*, 1972; Karlsson, Dahl & Marks, 1978).

It is often stated in papers that the diagnosis of DH was made on clinical and histological features and response to dapsone. This implies that DH has a characteristic clinical picture not seen in other disorders. Although the rash has certain features, and sites of predilection, they are not exclusive to DH. DH is grouped as one of the bullous disorders, but blisters also occur in diseases with which DH may be confused, e.g. eczema, pemphigoid, erythema multiforme, papular urticaria, and vasculitis. In addition, blisters may not always be seen and both Duhring and Brocq stressed the polymorphic nature of DH and emphasized that at any given time either erythematous, urticarial, or bullous lesions may predominate. Even if blisters are present they are often destroyed at an early stage by the continued scratching of the lesions. In the present study blisters were seen at some stage in seven of the sixteen IgA negative patients and in sixteen of the seventeen IgA positive patients. The distribution of the eruption is also variable in DH. The elbows, knees, buttocks and scapular region are usually given as the classical sites, but Alexander (1975b) in his review of 144 patients found involvement of the elbows in only 50%, buttocks in 44%, shoulders in 42%, and knees in 41%. A comparison of our patients shows that the elbows were involved in 71% of IgA positive patients compared to 25% IgA negative patients. The involvement of the knees was low in both groups being 29% in the IgA positive and 13% in the IgA negative group. The involvement of the scapular area was similar in both groups being approximately two-thirds. The buttocks were involved in 31% of the IgA negative and 53% of the IgA positive group. Thus, although the involvement of the elbows, knees and buttocks is higher in the IgA positive as opposed to negative group, the relatively low incidence in the positive group makes the distribution of the rash *per se* a relatively unreliable criterion for establishing the diagnosis.

The histology of the lesions was 'typical' of DH in six of the eight biopsies carried out in the IgA positive group, but only in two of the ten biopsies in the IgA negative group. Although it is accepted that there are histological features which are typical of DH they are not exclusive (Alexander, 1975c) to DH. The subepidermal blister is found in a number of other conditions and the micro-abscesses in the dermal papillae have also been reported in pemphigoid (Connor, Marks & Wilson Jones, 1972). However, the problem in many patients who have DH or suspected DH is to find a suitable lesion to biopsy. Ideally the biopsy should be taken from an early urticarial lesion surrounding a small blister. This is often difficult to find and probably accounts for the relatively low incidence of biopsies for histology compared to the other features of the disease in previous studies. Marks (1977) reported on the response to dapsone and IgA tests in a series of fifty-five patients, but only on the histology in thirty-four of these patients, and this appears to have been after challenge with potassium iodide to produce the lesions. Similarly Cooper & Cooke (1978) performed skin biopsies in only eleven of their twenty patients. Thus, in these two series the incidence of skin biopsies is similar to that reported in our study, which is just over 50% of patients.

A comparison of the small intestinal biopsies adds further evidence that the two groups of patients are different. All thirteen biopsies in the IgA negative group had a normal macroscopic appearance whereas six of the eleven biopsies in the IgA positive group had an abnormal macroscopic appearance as found in gluten sensitive enteropathy. This is a similar incidence to previous reports in DH (Fry *et al.*, 1972, 1974). Two of the thirteen IgA negative group had intra-epithelial lymphocyte counts above 300 (i.e. 355 and 357) which we had previously suggested was the upper limit of normal (Fry *et al.*, 1974). However, other workers Fergusson & Murray (1971) and Stevens *et al.* (1975) have reported intraepithelial lymphocyte counts above 300 in control subjects. The two patients with counts above 300 have now both been diagnosed as eczema and have stopped dapsone. Two of the IgA positive group had biopsies with a normal macroscopic appearance but with intra-epithelial lymphocyte counts of 588 and 445, which we consider to be above the upper limit of normal. Thus, eight of the eleven small intestinal biopsies in the IgA positive group show changes compatible with gluten sensitive enteropathy, whereas all the thirteen biopsies in the IgA group are probably within normal limits.

The mechanism of action of dapsone in controlling the eruption in DH is not known and it is now accepted that many other dermatoses, including subcorneal pustular dermatosis, pemphigus, cutaneous vasculitis, papular urticaria, and erythema elevatum diutinum may be controlled by dapsone in some instances. Thus, the clinical picture and response to dapsone are not sufficient to establish a diagnosis of DH. This view has recently been supported by Katz & Strober (1978). In addition to the above skin disorders it would also appear in certain instances that eczematous eruptions respond to dapsone. All these disorders may well be due to immunological mechanisms, but why only a very few patients with pemphigus, cutaneous vasculitis, or eczema respond to dapsone, unlike the majority with DH, is not known.

This long-term study suggests that there is a difference between patients with a dapsone responsive dermatosis who are IgA negative and those who are IgA positive. The follow-up suggests that the IgA positive patients are the ones who have true DH by the fact that the disease is likely to be persistent (and the IgA persists even in those few patients whose skin lesions clear spontaneously) and the fact there is evidence of gluten sensitive enteropathy in the IgA positive, but not in the IgA negative group. If the finding of IgA in the skin is accepted as necessary for the diagnosis of DH it probably explains the results in previous studies in which only the clinical (and in some patients histological) features and response to dapsone were used to make the diagnosis of DH. It is likely that in these studies a number of patients were included who did not have DH. This may explain the lower incidence of involvement of the elbows in the series reported by Alexander (1975b) in which it was found to be 50% compared to the 71% in our IgA positive series. Similarly the incidence of HLA-B8 was found to be 58% by Katz *et al.* (1972), 60% by White *et al.* (1973) and 68% by Gebhard *et al.* (1973), when IgA was not used as one of the criteria for the diagnosis of DH, but the incidence was 79% (Seah *et al.*, 1976) and 87% (Reunala *et al.*, 1976), a similar incidence to coeliac disease, when IgA was used as one of the diagnostic criteria. In our original series (Fry *et al.*, 1967) of twelve patients referred by other consultant dermatologists as having DH on clinical, histological and dapsone criteria, follow-up has shown that two patients did not have DH which alters the incidence of small intestinal and haematological abnormalities reported in that study.

Although the reason why IgA is deposited in the uninvolved skin in DH is not known, it is likely to be a feature of the disease whether it be due to an abnormality of IgA itself, of reticulin, or some other cause. The IgA may not be concerned with the production of the skin lesions because it is found in the uninvolved skin, and in patients in remission, and it may be an epiphenomenon. However, whatever the basic cause of DH it results in deposition of IgA in the skin, and the diagnosis cannot be considered established unless the IgA is found, although serial sectioning of the biopsy and further biopsies may be necessary to detect it in some patients.

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# Epidermolysis bullosa acquisita

## IMMUNOFLUORESCENCE, ELECTRON MICROSCOPIC AND IMMUNO-ELECTRON MICROSCOPIC STUDIES IN FOUR PATIENTS

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### SUMMARY

Four patients with the clinical picture of epidermolysis bullosa acquisita were investigated. Biopsies were taken from the involved and uninvolved areas of the skin and the immunohistochemical and microscopic changes were studied. Direct immunofluorescence showed deposition of IgG and C3/4 in a linear or notched pattern along the epidermal basement membrane in both the involved and the uninvolved skin. In addition IgA (3/4), IgM (1/4), C4 (3/4) and properdin (3/4) could be detected.

Indirect immunofluorescence revealed the presence of circulating antibodies against *inter alia* the epithelial basement membrane zone in one patient. Routine electron microscopy showed that the blister was situated in the dermis leaving the basal lamina in the roof of the blister. With immuno-electron microscopy using peroxidase-labelled antibody the *in vivo* deposition of IgG was observed just beneath the basal lamina in the dermis of both the perilesional and the uninvolved skin. These observations show that epidermolysis bullosa acquisita is a distinct entity, in which autoimmune mechanisms might possibly play a role.

Epidermolysis bullosa acquisita (EBA) is a rarely occurring acquired mechano-bullous disease. According to Roenigk, Ryan & Bergfeld (1971) the criteria for the diagnosis are: (1) clinical lesions of epidermolysis bullosa dystrophica; (2) adult onset of the disease; (3) negative family history for epidermolysis bullosa; (4) other bullous diseases excluded. The same authors stressed that in most cases concomitant systemic diseases are present, e.g. amyloidosis, colitis and enteritis. Histopathologically the blister formation is on the subepidermal level.

Since 1973 the presence of immunoglobulins (Ig) and complement (C) in the region of the epidermal basement membrane (BM) has been reported in EBA. In all reports IgG and C3 were demonstrated, but occasionally IgA, IgM and fibrin have been found in addition (Benedetto, Bergfeld & Guirguis, 1978). Linear, bullous pemphigoid-like pictures (Bruns, 1973; Metz, Frank & Metz, 1975), homo-

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geneous bandlike (Kushniruk, 1973; Gibbs & Minus, 1975) and even granular (Livden *et al.*, 1978) immunofluorescence (IF) patterns have been described. The normal skin, if examined, showed the same IF picture as the diseased skin. However, circulating antibodies against an antigen in the BM region have so far been detected in only one case (Provost *et al.*, 1979).

Woerdeman (1974) showed in an electron microscopic study that the blister formation takes place beneath the basal lamina, leaving this structure in the roof of the bulla. Recently this observation was confirmed (Benedetto *et al.*, 1978); others in addition described an electron dense, homogeneous, amorphous band, localized under the basal lamina in both the perilesional and uninvolved skin (Gibbs & Minus, 1975; Metz *et al.*, 1975). The latter authors stated that the number of anchoring fibrils is decreased in the involved skin. To our knowledge no immunoelectron microscopy studies on EBA have been reported in the literature.

In this study we report the results of IF, electron microscopic and immunoelectron microscopic studies in four patients with EBA. Moreover, an analysis has been made of the presence of complement factors in the skin and the preliminary results of the investigations on the incidence of circulating antibodies are given.

## PATIENTS AND METHODS

Four patients with EBA were investigated. The clinical data have been summarized in Table 1. The clinical diagnosis was supported by routine histopathology. Biopsies for IF and immunoelectron microscopy were taken from the perilesional and uninvolved skin (extensor aspect of the forearm), biopsies for routine electron microscopy from the edge of a fresh blister.

Direct IF studies were performed as described previously (Nieboer & Kalsbeek, 1978). In addition to the routine IF procedure (IgG, IgA, IgM, IgD, IgE, C3/4; albumin, fibrinogen), unfixed cryostat sections were incubated with unlabelled rabbit sera directed against the human C-factors C1q, C3B, C3c, C3d, C4, C5 and C3-proactivator (C3PA) in the first incubation step and with horse anti-rabbit Ig-FITC (prot. conc. 11·6 mg/ml, F/P ratio 1·8, dil. 1:70) in the second step. For detection of properdin a goat anti-properdin serum (batch no. 1080 L1, dil. 1:40, Flow laboratories) was used, followed by incubation with a rabbit anti-goat Ig-FITC conjugate (prot. conc. 8 mg/ml, F/P ratio 3·1, precipitation titre 1:32, dil. 1:50, absorbed to human Ig). Normal sera from rabbit and goat respectively served as negative controls in the first step. All antisera, except the goat anti-properdin serum, were commercially obtained from the Central Laboratories of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

Indirect IF was carried out with dilutions of the sera from the EBA patients, a bullous pemphigoid (BP) patient and a healthy individual. Unfixed cryostat sections from human normal skin and oral mucosa, guinea-pig lip and rat oesophagus, lung, stomach, colon, spleen, liver, kidney hilum and striated muscle were used as substrates. Sheep anti-human IgG-FITC (unitage 4, F/P ratio 1·37, dil. 1:20, prepared in our laboratory) was used in the second step.

For the routine electron microscopy the tissue was dehydrated and embedded in a mixture of Epon and Araldite, after fixation in 2% cacodylate-buffered glutaraldehyde and postfixation in 1% osmium-tetroxide. The sections were counterstained with uranyl acetate followed by lead citrate and examined on a Siemens Elmiscope 51 and a Philips EM 301.

For the immunoelectron microscopy a horseradish peroxidase (HRP) conjugate was prepared from rabbit antibodies directed against human IgG (H+L), isolated by immunoabsorption (Boorsma & Streefkerk, 1978) and HRP (RZ 3·0, Boehringer Mannheim, W. Germany) by the two-step glutaraldehyde method (Avrameas & Ternynck, 1971). For the coupling reaction monomeric activated HRP (Boorsma & Streefkerk, 1976a) was used and the conjugate was purified from unconjugated

TABLE 1. Clinical data of the four patients with epidermolysis bullosa acquisita

| No. | Sex | Year of birth | Age at onset | Localization |  | Clinical symptoms |  |  |  | Porphyrins | Associated conditions | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Skin | Mucosal | Blisters | Milia | Atrophy | Nail dystrophy |  |  |  |
| 1 | F | 1897 | 74 | Hands, knees | – | + | + | + | + | – | – | No response to vitamin E, dapsone |
| 2 | M | 1928 | 42 | Hands, knees | – | + | + | + | + | – | – | No response to vitamin E, dapsone |
| 3 | F | 1941 | 37 | Hands, knees, feet, elbows | – | + • | + | + | + | – | – | No response to vitamin E, dapsone, zinc sulphate, retinoid |
| 4 | F | 1903 | 75 | Hands, feet | – | + | + | + | + | – | – | None |

TABLE 2. Routine direct immunofluorescence of perilesional and normal skin: staining of the BM region

| Patient no. | IgG | IgA | IgM | IgD | IgE | C | Fibrin | Albumin | Thioflavin-T |
|---|---|---|---|---|---|---|---|---|---|
| 1 | +L | ±L | – | – | – | +L | – | – | – |
| 2 | +L | – | ±G | – | – | +L/G | – | – | – |
| 3 | +L | +L | +F/G | – | – | +L | – | ±L | – |
| 4 | +L | ±L | – | – | – | +L/F/G | +F | – | – |

L: linear; G: granular; F: fibrillar.

HRP and free antibodies by gel chromatography on Ultrogel AcA-44 (Boorsma & Streefkerk, 1976b). In a similar way sheep antirabbit IgG-HRP was prepared as control.

Biopsies were mounted in Tissue-tek II O.C.T. compound (Miles, Naperville, Illinois), immediately snap frozen and stored in liquid nitrogen. The further processing has been described in detail elsewhere (Boorsma, 1977). Briefly, 8 μm cryostat sections were prefixed for 2 min in periodate-lysine-paraformaldehyde (PLP) fixative (paraformaldehyde concentration 0·125%) according to McLean & Nakane (1974), incubated with the rabbit anti-human IgG-HRP conjugate (antibody concentration 25 μg/ml) for 1 h, postfixed for 2 min with 0·5% glutaraldehyde in 0·05 M phosphate buffer, pH 7·4, incubated with diaminobenzidine/$H_2O_2$, osmicated for 2 min, dehydrated and embedded in epon-araldite. Ultrathin sections were cut with a diamond knife. As a specificity control the same procedure was carried out with the sheep anti-rabbit IgG-HRP conjugate.

## RESULTS

### *Direct immunofluorescence*

IgG and C3/4 (Table 2) were found in all cases, in both the perilesional and the clinically normal skin, in a linear or notched pattern (Figs 1–3) along the epidermal and follicular BM. Interrupted linear, fibrillar and granular patterns were usually seen only next to the blister and below it. The walls of the vessels did not contain granular deposits. In three cases IgA could be demonstrated in the same pattern as IgG and C3/4 but less extensive, while IgM was present in one case. Fine fibrillar deposits of fibrin along the BM were present in one case in the perilesional skin only. The presence of C3, especially C3c and C3d (Fig. 2), appears to be a constant feature (Table 3). In addition both C4 and properdin (Fig. 3) were detectable in three of the four cases, whereas C1q was found in only one case. C3PA was not found in a specific pattern.

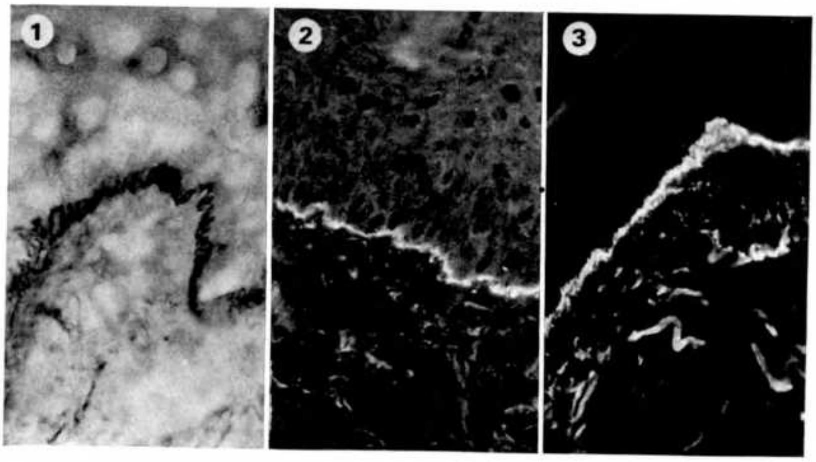


FIGURE 1. Perilesional skin. Linear and notched picture of the BM (HRP labelled anti-human IgG, ×320 oil).

FIGURE 2. Perilesional skin. Positive BM (anti-properdin, ×200).

FIGURE 3. Uninvolved skin. Positive BM (anti-C3d, ×200).

### *Indirect immunofluorescence*

The preliminary results of the indirect IF studies indicate that several IgG auto-antibodies can be

TABLE 3. Incidence of the complement-factors along the epidermal BM

| Patient no. | C1q | C4 | C3B | C3c | C3d | C5 | Properdin | C3PA |
|---|---|---|---|---|---|---|---|---|
| 1 | — | ± | + | + | + | — | + | — |
| 2 | — | ± | + | + | + | ± | — | — |
| 3 | ± | — | — | + | + | — | ± | — |
| 4 | — | + | ± | + | + | + | + | — |

found in the sera (Table 4). The reaction of the antibodies with antigen(s) in the BM region of stratified squamous epithelium resulted in a linear picture together with a staining of the basal parts of the basal cell cytoplasm. The positive reaction of two sera with striated muscle consisted of a homogeneous staining of short parts of the fibres, suggesting a binding to the sarcolemma. One serum showed a weak positive reaction with the BM of the parietal layer of Bowman's capsule in the rat kidney. The negative controls gave no positive results; the bullous pemphigoid serum reacted with the BM of the stratified squamous epithelia in a sharp linear pattern.

TABLE 4. Incidence of circulating IgG antibodies against tissue antigens

| | Human | | Guinea-pig | | Rat | | | |
|---|---|---|---|---|---|---|---|---|
| Patient no. | Skin | Oral mucosa | Lip | ANA | Oesophagus | Kidney | Striated muscle | Lung, spleen liver, stomach colon, pyelum |
| 1 | — | — | — | — | — | — | — | — |
| 2 | +* | +* | +* | + | +* | +† | +‡ | — |
| 3 | — | — | — | — | +* | — | — | — |
| 4 | — | — | — | + | — | — | +‡ | — |

ANA: anti-nuclear antibodies

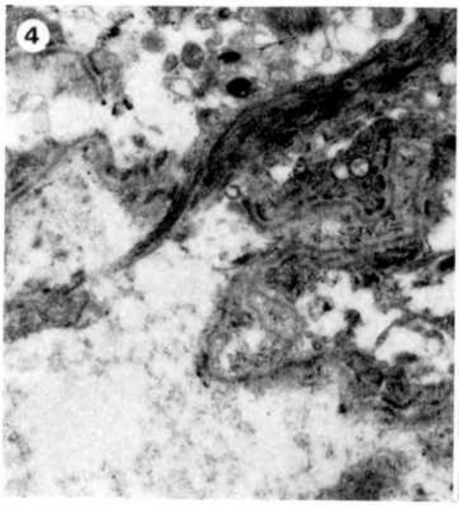
* BM region; † BM parietal layer Bowman's capsule; ‡ short fragments: sarcolemma?

### *Routine electron microscopy*

The bulla appeared to be localized in the superficial layers of the dermis (dermolytic blistering), the roof of the bulla being formed by the basal lamina. The basal lamina showed focal destruction through which tonofibrils ended freely in the bulla. In the roof of the bulla fragments of collagen fibres and amorphous materials were present. There was oedema of the stratum basale and degeneration of the keratinocytes (Fig. 4).

### *Immunoelectron microscopy*

From Fig. 5 it is clear that the pre- and postfixation methods used in our technique result in a good preservation of the ultrastructure of the tissue. The number of anchoring fibrils appeared to be decreased in the perilesional skin (Fig. 5). Figures 6–9 show that the electron dense reaction product, which indicates the site of the immunoglobulin deposits, is localized as an amorphous, fine granular band just beneath the basal lamina.



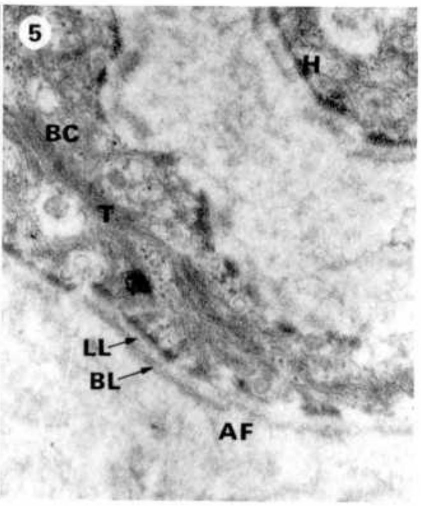
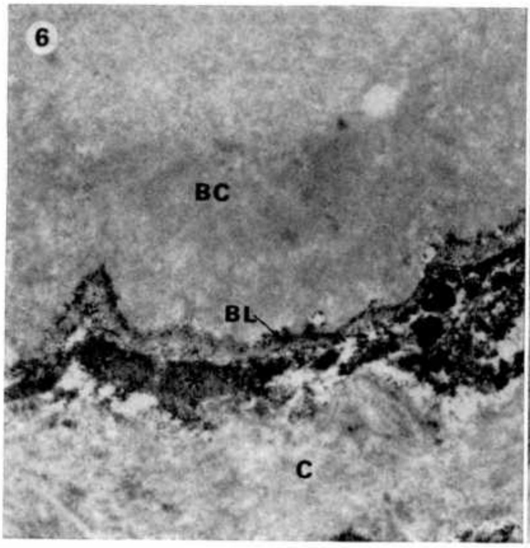


FIGURE 4. Routine electron microscopy of bulla. Dermal localization of the bulla, the roof of the bulla being formed by the basal lamina. Focal destruction of the BM through which tonofibrils end freely in the bulla. Degeneration of the keratinocytes in the stratum basale (× 35,000).

FIGURE 5. Perilesional skin. A frozen 8 μm section is mounted on an object slide and fixed in 0·5% glutaraldehyde, in 0·05 M phosphate buffer pH 7·4. No immunological incubations. Counterstained with uranyl acetate and lead citrate. AF = anchoring fibrils; T = tonofilaments in basal cell (BC); BL = basal lamina; LL = lamina lucida; H = hemidesmosome (× 78,000).



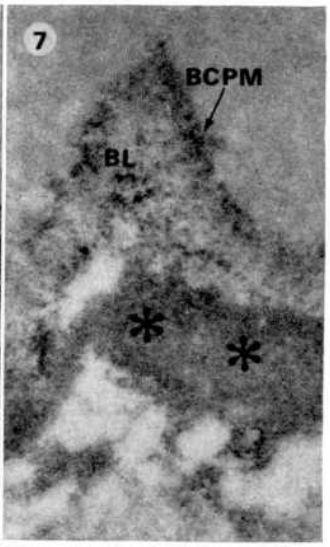
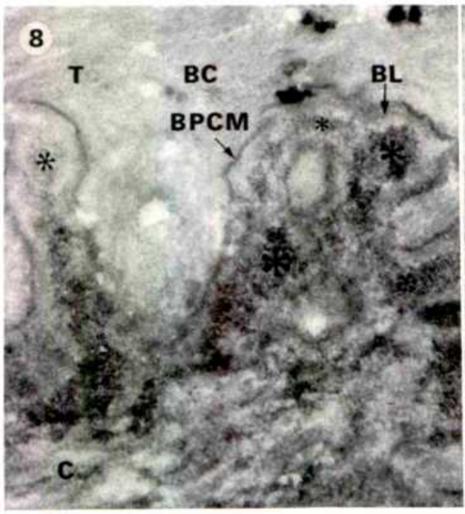


FIGURE 6. Perilesional skin. PLP fixative. Incubation with HRP-labelled rabbit anti-human IgG. Electron dense reaction product (asterisk) is localized just beneath the basal lamina (BL). BC = basal cell; C = collagen fibres. No counterstain (× 17,500).

FIGURE 7. Detail of Fig. 6. Heavy deposition of electron dense reaction product (asterisk). BCPM = basal cell plasma membrane; BL = basal lamina. No counterstain (× 74,500).



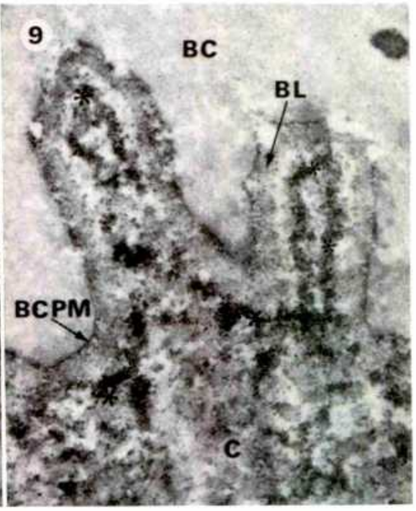


FIGURE 8. Uninvolved skin. PLP fixative, HRP-labelled rabbit anti-human IgG. Electron dense reaction product (asterisk) is localized just beneath the basal lamina (BL). BC = basal cell; BCPM = basal cell plasma membrane; C = collagen fibres, T = tonofilaments. Counterstained with uranyl acetate (× 16,000).

FIGURE 9. Uninvolved skin. PLP fixative, HRP-labelled rabbit anti-human IgG. Electron dense reaction product (asterisk) is localized on the dermal side parallel to the basal lamina (BL). BC = basal cell; BCPM = basal cell plasma membrane; C = collagen fibres. No counterstain (× 32,500).

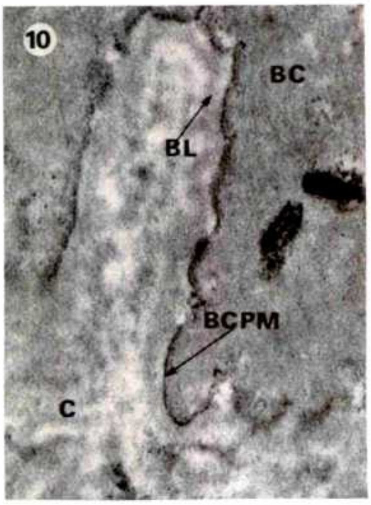


FIGURE 10. Uninvolved skin. PLP fixative, HRP conjugated sheep anti-rabbit IgG. Note absence of electron dense reaction product in the specific loci (i.e. just beneath the basal lamina) as compared to Fig. 9. BC = basal cell; BCPM = basal cell plasma membrane; BL = basal lamina; C = collagen fibres. No counterstain (× 32,500).

Although the lamina lucida is not so clearly distinctive, no reaction product was found at this site. There appeared to be a small space between the basal lamina and the reaction product.

In the perilesional skin (Figs 6 and 7) this product showed a more massive and irregular appearance than the pattern in clinically normal skin (Figs 8 and 9). The specificity of the reaction is proven in Fig. 10, in which no reaction product is visible. However, it must be noted that in the same picture a band of fine granular electron dense material is present, showing the same localization as the immunoglobulins.

## DISCUSSION

The clinical and laboratory data of our four patients point unequivocally to the diagnosis EBA. It is of interest that our patients did not show the high incidence of associated systemic diseases as mentioned by Roenigk *et al.* (1971). It is well known that several pathological processes can exist in older patients with no other common feature than the aging of the organism. In this respect it is of importance that the two relatively young patients of our series were otherwise in perfect health. The occurrence of associated systemic diseases in the diagnosis EBA therefore is not always evident.

The direct IF pattern in EBA is not entirely characteristic. In comparison with bullous pemphigoid the more fibrillar and notched appearance of the BM in EBA might be an indication, but in most cases the clinical and other laboratory parameters will be necessary for the correct diagnosis. The differentiation from porphyria cutanea tarda is possible because in EBA the linear fibrillar pattern of the BM is more sharply defined in the absence of the strongly positive homogeneous staining of the walls of the vessels, well known in porphyria (Cormane, Szabo & Tio, 1971). Lastly, the IF method may be a useful tool for the distinction between EBA and epidermolysis bullosa dystrophica. In three patients with this disease we observed only a non-specific IF picture but the occurrence of IgG and C along the BM has been described in one case (Metz *et al.*, 1975).

In routine electron microscopy in EBA the localization of the bulla in the superficial layer of the dermis clearly differs from that in bullous pemphigoid. Most authors agree that in bullous pemphigoid the blister is localized in the intermembrane space with the basal lamina on the floor of the blister. It is important to examine fresh blisters.

It has been pointed out (Caputo, Bellone & Crosti, 1973; Woerdeman & de Jong, 1971) that the stage in which biopsies are performed is important and that this may explain many controversial findings reported in literature. Possibly it also explains that in the inflamed blisters of bullous pemphigoid the basal lamina is destroyed (Schaumberg-Lever, Orfanos & Lever, 1972). Electron microscopic studies on blister formation in cicatricial pemphigoid have led to controversial results. In oral lesions the basal lamina has been found partly in the roof and partly in the base of the blister (Susi & Shklar, 1971), also at the blister base and thirdly destroyed within blisters (Caputo *et al.*, 1973). Therefore it may be impossible in some cases to differentiate between EBA and cicatricial pemphigoid by routine electron microscopy.

At the ultrastructural level the localization of the Ig deposits in EBA differs from that in bullous pemphigoid. In the latter the localization is on the basal cytoplasm membrane above the basal lamina (Holubar *et al.*, 1975). In EBA where there is an intact dermal epidermal junction, IgG is deposited beneath the basal lamina. It consists of a granular band parallel to this structure at a rather equal distance. This regular pattern is remarkable because in these layers of the dermis a known morphological structure is usually not present.

However, as already mentioned, an electron dense band with the same localization of IgG can be found in EBA. The nature of this band is as yet unknown. When the dermo–epidermal junction is disrupted irregular deposits of IgG take the place of the regular picture. In this respect the results of the immunoelectron microscopy are in accordance with those of the direct IF.

From our results it appears that there is a clear distinction between EBA and bullous pemphigoid. Differentiation between EBA and cicatricial pemphigoid seems to be more difficult. In our opinion clinical and histopathological features point to EBA as a distinct entity. However, direct and indirect IF give approximately the same results in both diseases. Although the electron microscopic picture in EBA is constant, it may be impossible in some cases to differentiate between EBA and cicatricial pemphigoid. Immunoelectron microscopic data, which are lacking in cicatricial pemphigoid, will contribute towards resolving this problem.

Several arguments can be brought forward in favour of the assumption that the presence of Ig and C reflects an antigen-antibody reaction, e.g. the constant findings in the direct IF and immunoelectron microscopy as well as the activation of the C-system by both the classical and the alternative pathway. The ultrastructural localization indicates that most probably the supposed antigen is of mesodermal origin: reticulin, collagen, elastin (Kobayasi, 1977) or ground substance. Our preliminary results of the indirect IF, however, do not clearly point to a reaction of circulating antibodies with non-denatured connective tissue antigens. In this respect it is noteworthy that Lazarus (1972) found circulating antibodies against collagenase-denatured collagen in four out of six patients with epidermolysis bullosa dystrophica. Similar experiments with sera from EBA patients are in progress in our laboratory. An alternative explanation might be that extravasated Ig and C molecules are trapped by the electron dense band beneath the lamina densa and bound in a non-immunological way.

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## ADDENDUM

After completion of our manuscript two articles concerning epidermolysis bullosa acquisita were published: Dahl, M.C.G. & Cook, L.J. (1979) Lesions induced by trauma in pemphigoid. *British Journal of Dermatology* **101**, 469 and Dahl, M.C.G. (1979) Epidermolysis bullosa acquisita—a sign of cicatricial pemphigoid? *British Journal of Dermatology*, **101**, 475.

Although we agree that there are similarities between EBA and cicatricial pemphigoid, we believe that our observations show that our patients belong to a distinct entity (EBA) different from cicatricial pemphigoid.

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# Desquamative gingivitis: a form of cicatricial pemphigoid?

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## SUMMARY

Desquamative gingivitis, a sub-epithelial vesiculo-bullous disease involving the gingiva, has been shown to have positive direct immunofluorescent findings similar to those of cicatricial pemphigoid. However, the lack of severe scarring, limitation of lesions to the anterior portion of the mouth and the tendency toward limitation of lesions to the oral mucosa over long periods of time in desquamative gingivitis, suggest that desquamative gingivitis and cicatricial pemphigoid are distinct and separate entities.

Desquamative gingivitis (DG) is a chronic inflammatory disorder primarily involving the gingival epithelium of post menopausal women. The gingivae are usually erythematous with a 'polished appearance' and a white friable membrane that peels away with minimal trauma leaving a raw denuded surface.

Intact bullae may occasionally be seen since they form subepidermally and are not inordinately fragile. Even very gentle massage of apparently unaffected mucosa may induce bullae and/or desquamation (intra-oral Nikolsky's sign). Pain and tenderness although often presenting symptoms may not be marked and produce variable degrees of morbidity. Scarring is not a prominent feature.

Although first recognized by Tomes (1897), Prinz (1932) characterized the disorder and named it 'chronic diffuse desquamative gingivitis'. Subsequent authors writing primarily in the dental literature considered DG to be a disease *sui generis*. Shklar & McCarthy (1960) pointed out that DG was not a single disease but rather a heterogenous group of disorders including cicatricial pemphigoid, erosive lichen planus and pemphigus vulgaris which may be clinically indistinguishable. Although clinically similar, histological and more recently, immunological differences have been recognized which permit separation into distinct entities.

We recently became interested in DG when one of our patients with this condition showed positive direct immunofluorescence (DIF) suggestive of pemphigoid on an oral biopsy. Rogers, Sheridan & Morgan (1976) have reported a group of patients with DG evolving into cicatricial pemphigoid (CP).

We have subsequently studied a group of six patients with DG, histologically and by conventional

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immunofluorescence (IF) techniques*, selected from a group of fifty cases diagnosed as subepidermal bullous disease of the mouth (since 1972) in the files of the Oral Pathology Department of Emory University Dental School, and present our findings and tentative conclusions. All six of our cases, clinically typical DG, showed positive basement membrane zone (BMZ) staining compatible with pemphigoid.

## CASE HISTORIES

### *Case 1*

A 58-year-old woman was first treated in 1963 for a psoriasiform eruption of the scalp and left palm. In 1973 her dentist noted white areas on her gingivae, biopsied one, and diagnosed oral psoriasis.

On examination the entire maxillary and mandibular gingivae were erythematous and tumid-appearing with a friable lacy membrane which peeled off with minimal trauma. The lower alveolar gingivae in the premolar and molar areas were more severely involved, possibly secondary to trauma. Bullae have been apparent at times.

An ulcer occurred on the hard palate which healed with slight scarring (Fig. 1). Otherwise, scarring was minimal. She has had chronic asymptomatic 'conjunctivitis' but no other evidence of ocular disease.

Histological examination of a biopsy of gingival mucosa showed a sub-epidermal bulla with complete separation of the epithelium from the underlying lamina propria (Fig 2). There was no degeneration of the epithelium and a mild chronic inflammatory cell infiltrate was present in the connective tissue. The findings were consistent with pemphigoid.

Direct immunofluorescence of a gingival biopsy showed deposition of IgG and $C_3$ at the basement membrane zone (BMZ) in a granular pattern. Indirect immunofluorescence (IIF) was negative. Antinuclear antibody (ANA) has been positive in titres of 1:20, 1:80, 1:160 (speckled pattern) (Table 1).

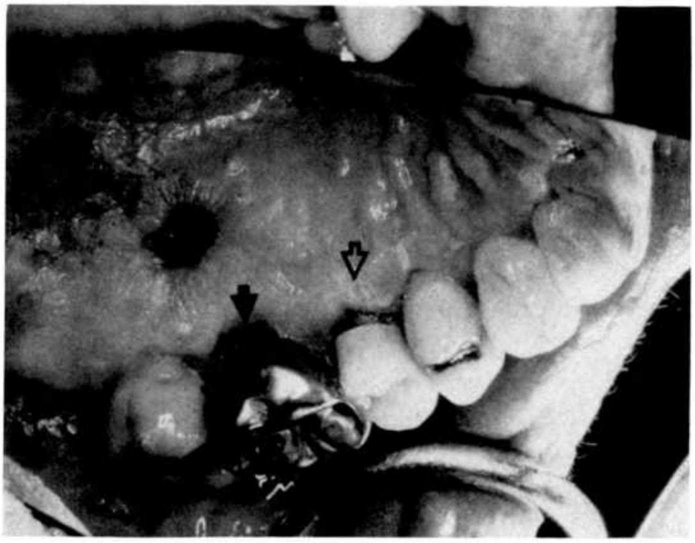

FIGURE 1. The marginal gingiva shows erythema (solid arrow) and a white friable membrane (open arrow). A healing ulcer is present on the hard palate (Case 1).

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* Performed by Immunofluorescent Testing Service, Buffalo, New York.

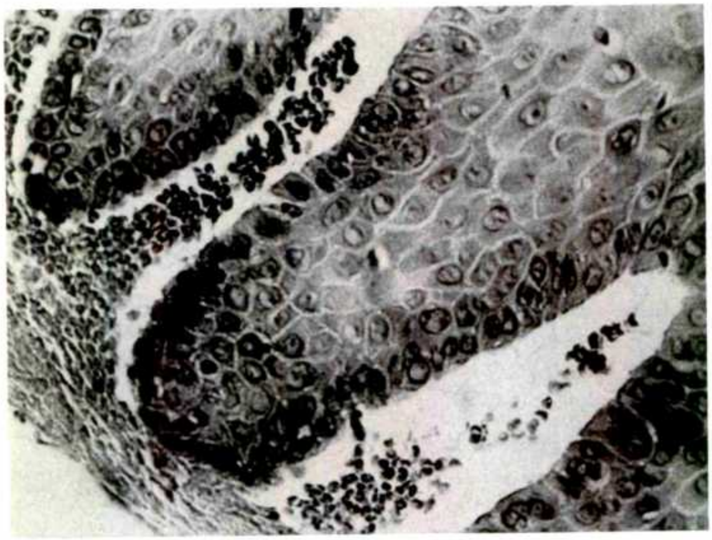

FIGURE 2. The intact basal layer of epithelial cells is seen in the roof of this subepithelial bulla. Extravasated erythrocytes are present (Case 1) (× 160).

TABLE 1. Summary of clinical and immunofluorescence data

| Patient | Age (years) | Race and Sex | Biopsy Site | DIF-BMZ Immuno Glob. | | | | | Pattern | IIF | ANA | Sites involved |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | G | A | M | C3 | Fib. | | | | |
| 1.PM | 59 | WW | Oral<br>Lesion | + | – | – | + | – | Granular | neg | 1:20<br>1:160<br>1:80 | Gingiva<br>palate<br>Eye ?? |
| 2. VS | 68 | WW | Oral<br>Normal | + | – | – | + | – | Linear | neg | N.D. | Gingiva<br>vulva<br>nose |
| | | | Lesion | + | + | + | + | – | Linear | | | |
| | | | Genital<br>Normal | + | – | – | + | – | Linear to granular | | | |
| 3. PA | 25 | WW | Oral<br>Lesion | + | + | + | + | + | Linear and Fibrillar | IC 1:80<br>BMZ 0 | neg | Gingiva |
| 4. MS | 71 | WW | Oral<br>Lesion | + | – | – | – | + | Linear | neg | N.D. | Gingiva |
| 5. DC | 35 | WM | Oral<br>Lesion | – | – | + | – | + | Linear | neg | N.D. | Gingiva<br>buccal<br>penis |
| 6. MP | 34 | WW | Oral<br>Lesion | – | – | + | – | + | Granular | neg | neg | Gingiva<br>vulva |

N.D. = not done.

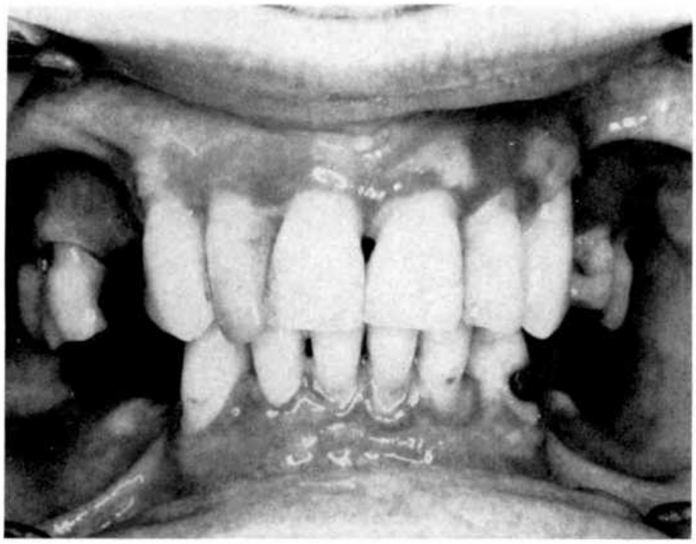

FIGURE 3. Erythema and collapsed bullae and fibrin membranes overlying ulcerative areas are shown on the upper and lower gingiva (Case 2).

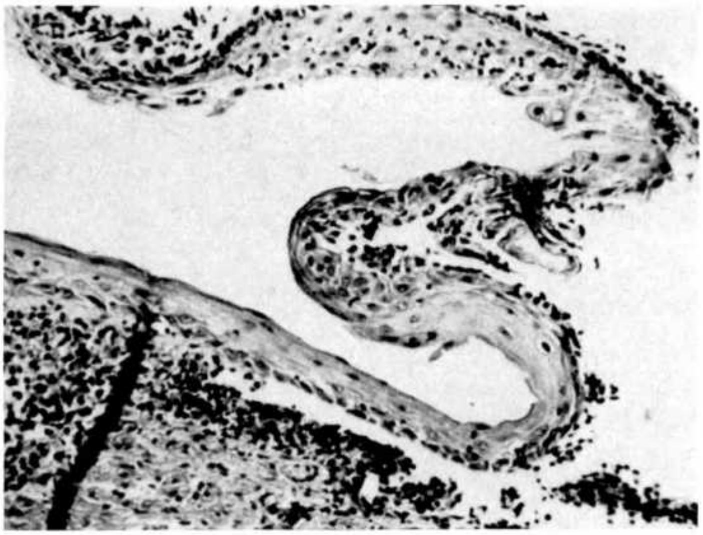

FIGURE 4. A subepithelial separation is present with a chronic inflammatory cell infiltrate in the lamina propria in this oral biopsy (Case 2) (× 40).

*Case* 2

A 68-year-old woman has had DG for 3 years, genital itching for a number of years and an irritation in the nose.

There was diffuse desquamation and friability of the gingival mucosa (Fig. 3) with intact bullae. Superficial ulcers were noted on the right side of the nasal septum and on the posterior vaginal wall. No significant scarring was present. Her eyes were unaffected. She was treated with a topical corticosteroid (fluocinolide acetonide gel). Oral and perineal biopsies (Fig. 4) were consistent with pemphigoid. Direct immunofluorescence on normal appearing oral mucosa showed positive IgG and C3 in a linear band at the BMZ. Lesional oral mucosa was positive for IgG, IgA, IgM, and C3 in a linear band. Genital perilesional skin was positive for IgG and C3 in a 'linear to granular pattern'. Indirect immunofluorescence was negative (Table 1).

*Case 3*

A 27-year-old white woman, 5 months pregnant, complained of desquamating tender gingivae for almost a year. The gingivae were erythematous and a white membrane was present on many of the interdental papillae. There was no significant scarring. The remainder of the oral tissues were normal. There was no involvement of the eyes or genitalia. Routine H & E sections of gingiva showed early subepidermal vesiculation compatible with pemphigoid.

Direct immunofluorescence of lesional and perilesional gingivae showed IgG, IgA, IgM, fibrinogen and C3 in the BMZ in a linear fibrillar pattern. Indirect immunofluorescence was negative for BMZ antibodies but positive at a titre of 1:80 for intracellular antibodies. Antinuclear antibody determination and a LE preparation were negative (Table 1).

*Case 4*

A 71-year-old white woman had a sore mouth for over a year. Gingival erosions were present throughout the mouth but no other sites were involved. No genital or ocular lesions were present. A subepithelial bulla was seen on biopsy.

Direct immunofluorescence of perilesional gingiva showed IgG and fibrinogen desposits in the BMZ in a linear pattern. Indirect immunofluorescence was negative for BMZ and intercellular antibodies (Table 1).

*Case 5*

A 35-year-old male had a 4 year history of intermittent superficial ulcers involving the mouth and penis. At the time of examination there were erosions of the gingiva and buccal mucosa. Nikolsky's sign was positive. However, no penile or other lesions were present. A biopsy showed histological features of pemphigoid.

Direct immunofluorescence of the lesional gingiva showed IgM and fibrinogen deposits in a linear pattern in the BMZ. Indirect immunofluorescence was negative (see Table 1).

*Case 6*

A 34-year-old female presented with slightly painful gingival erosions which had been occurring for several years. There was no evidence of significant scarring. Vaginal erosions occurred, following extended intercourse. A lesion on the upper eyelid was biopsied and showed an early subepidermal bulla. Biopsy of a gingival lesion showed a subepithelial bulla.

Direct immunofluorescence of gingiva was positive for IgM and fibrinogen with a granular pattern in the BMZ. Indirect immunofluorescence, ANA and LE preparations were negative (Table 1).

## DISCUSSION

Desquamative gingivitis has been an imprecisely defined clinical entity which usually affects the gingivae of older women; however, younger women and even men may be affected.

The exact incidence is not known but is probably greater than the number of reports in the literature would indicate since we have been able to identify fifty cases in a 6-year period by examining approximately 13,000 accessions in our files. Most cases are probably diagnosed by dentists. Dermatologists may not be as familiar with this disorder since it is only briefly mentioned in textbooks of dermatology and few case reports appear in the recent dermatological literature.

The term DG has classically been used in a descriptive manner for a heterogeneous group of clinical disorders characterized by subepidermal, or rarely intraepidermal, bullae. Since the bullae of DG may show features ordinarily seen in CP, bullous pemphigoid (BP), lichen planus (LP) or other less well characterized subepidermal blistering diseases further classification by light microscopy is not feasible. We have utilized immunological techniques and have identified a fairly homogeneous subset of cases with BMZ immunfluorescence resembling BP and CP. These patients do not appear to have BP since none has had circulating BMZ antibodies or significant involvement of non-mucosal surfaces. They also differ from CP since scarring has been minimal, no significant eye involvement has occurred and lesions have spared the posterior pharynx. One patient (Case 2) has had nasal mucosal ulcers and three patients genital ulcers for years with minimal scarring. The lack of severe scarring in our patients, who have been followed from 2 to 5 years, may be related to the duration of the disease. We feel however that the absence of significant scarring is a characteristic of the malady we are reporting. It seems unlikely that the basic nature of this disease changes from a non-scarring or minimally scarring process to a severe cicatricizing deforming one. One of our cases (PM) has been followed for 5 years without evidence of increased scarring or extension of her disease beyond the oral mucosa, although extension from the gingiva onto the palate occurred.

A group of fourteen patients with a similar presentation has recently been reported by Forman & Nally (1977) under the cumbersome term 'oral nondystrophic bullous eruption mainly limited to the gingiva: a mechanobullous response'. Patients had recurrent blistering limited entirely to the mouth. The average age was 52 years and there was a female predominance. The labial gingivae were predominantly affected and the bullae arose spontaneously and could be provoked by trauma. No atrophy, shrinkage or bridging of sulci occurred even after 14 years in one patient. No extraoral lesions occurred. Biopsies showed subepithelial bullae; however DIF was positive in only two cases of the five examined. One showed C3 alone in the BMZ and the other IgG plus C3. Circulating antibodies were present in the one patient to intercellular epithelial substance and in another to epithelial cells. Topical corticosteroids were prescribed for all with minimal effect. One patient was treated with 20 mg of prednisone daily and his condition worsened. Patients were observed from 1 to 7 years and in ten (of fourteen) the condition gradually remitted. Although these patients clinically resemble ours, only two are immunologically similar. The absence of positive DIF in three of five, however, suggests that this group of cases does not represent a pathologically homgeneous entity.

The exact relationship of DG to CP and BP is unclear. Rogers *et al.* (1976) initially classified six out of their seven cases of DG as 'possible CP'. Five developed 'unequivocal CP' in the subsequent 3 years and one proved to have LP; however the clinical descriptions of these cases are not reported in detail. The seventh case was classified as DG since no extension to extragingival sites occurred during the follow-up period. Extragingival extension was apparently their prime criterion for evolution of DG to CP while little attention was given to the severity of scarring present. In our opinion significant scarring is the *sine qua non* for the diagnosis of CP.

All six of Rogers *et al.* (1976) patients in the DG–CP group showed positive DIF. This prompted them to conclude that DG and CP were one disease. If this is true there is little need to retain the term DG since CP limited to the gingiva would suffice. However, we do not believe that the DIF data alone are sufficient evidence to substantiate this concept. We believe that scarring is the primary clinical criterion for the diagnosis of CP. Since DG has little or none it appears to represent a disease apart from CP in spite of their similar histology and immunofluorescence.

Rogers *et al.* (1976) appropriately stress the importance of histological and immunological evaluation of patients presenting with oral mucosal bullae or desquamation. Direct and indirect immunofluorescence are mandatory for precise classification but must be correlated with the clinical findings.

Bean *et al.* (1972), Person, Rogers & Jordan (1977) and others have now demonstrated circulating anti-BMZ antibodies in patients with CP. They suggest that CP is closely related to, if not part of, the same disease spectrum as BP. Regardless of the validity of this concept, DG appears to have features which distinguish it from either, such as lack of profound scarring and sparing of the posterior pharyngeal and oesophageal mucosa.

### *Treatment*

We know of no safe and effective treatment for DG. We have used topical corticosteroids to treat our patients and our results, like Forman and Nally's (1977), have been unsatisfactory. Occlusion of these agents by prosthesis may enhance their effectiveness. Person & Rogers (1977) have reported the successful use of sulphones (DDS) and sulphapyridine in the treatment of BP, based on the observation that these drugs are effective in non-infectious diseases in which the accumulation of neutrophils occurs, and on the work of Barranco (1974) demonstrating lysosomal stabilization by dapsone.

Brody & Pirozzi (1977) have successfully treated a patient with CP with cyclophosphamide but we do not feel that the inherent risk associated with the use of this drug is justified in DG.

## CONCLUSIONS

Our experience to date based on a small number of cases suggests that DG, while grossly similar histologically and immunologically to BP and CP, has profound clinical differences, such as lack of scarring, anterior localization of disease and absence of ocular involvement. We feel that these differences are so basic that DG should continue to be classified separately from BP and CP. Rather than coining a new name, we prefer the term DG to describe this clinicopathological condition until the exact aetiology and pathogenesis of these diseases are better understood.

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# The mitotic index in psoriatic plaques and their response to PUVA therapy

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## SUMMARY

The mitotic index of pretreatment plaques of chronic psoriasis was determined before treatment with PUVA. There was a wide variation in mitotic indices in different patients.

Treatment was given with UV-A 2 h after oral ingestion of 8-methoxypsoralen (8-MOP) two or three times per week, with increasing doses of UV-A. Patients were considered cleared if there was 95% clearing of all psoriatic plaques.

There was no correlation between the mitotic index and the response to treatment, as measured by either the number of joules administered or the number of treatments until the lesions cleared. This suggests that the action of PUVA in psoriasis is not dependent on the amount of increase in epidermal proliferation.

Enhanced epidermal proliferation in psoriasis is evidenced by increased numbers of epidermal cell mitoses. Epidermal cell kinetic studies have demonstrated increased DNA synthesis and rapid proliferation. There is a decrease in the transit time of epidermal cells as demonstrated by the injection of tritiated amino acids (Porter & Shuster, 1967). This rapid proliferation and decreased transit time of psoriatic epidermal cells is manifested clinically by thickening and scaling of the affected skin.

Photochemotherapy with psoralen and ultraviolet A radiation (PUVA) has been shown to be effective in the treatment of psoriasis (Parrish, Fitzpatrick & Tanenbaum, 1974). Psoralens react with the pyrimidine bases of intertwining strands of DNA, and under the influence of UV-A irradiation (320–400 nm) form covalent bonds (Musajo, Rodighiero & Dall'Acqua, 1967; Dall'Acqua *et al.*, 1974). These photo-adducts interfere with DNA synthesis and cell multiplication (Epstein & Fukuyama, 1975); this is a postulated mechanism of action of PUVA therapy in psoriasis.

After PUVA treatment there is a decrease in the labelling index representing DNA synthesis of epidermal cell nuclei, as shown by autoradiography (Walter *et al.*, 1973). The treated, regressed psoriatic plaques show a return to normal of previously high epidermal mitotic counts, while there is no comparable change in the growth activity of simultaneously treated, clinically uninvolved skin. This differential action upon involved and uninvolved epidermis is seen in many effective therapeutic agents in psoriasis. It has been proposed that there is a specific direct inhibitory action upon the

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epidermis of psoriatic plaques as opposed to uninvolved areas based upon the rapid growth activity (Weinstein & Frost, 1968). If this were the case, a difference in response to therapy, dependent upon the epidermal growth activity at the onset of treatment, would be expected. There is a striking difference in epidermal growth activity in psoriatic plaques of different patients, as measured by the mitotic index (Cox & Watson, 1972; Fry & McMinn, 1968). We have found this to be the case in psoriatic plaques of patients about to be subjected to PUVA therapy.

This study was undertaken in order to test the hypothesis that the more rapidly proliferating epidermal cells of psoriasis are more sensitive to treatment with PUVA by determination of any correlation between the pretreatment mitotic activity in a representative psoriatic plaque and the response to PUVA treatment.

## MATERIALS AND METHODS

Patients with extensive psoriasis who were selected for PUVA treatment had 3 mm punch biopsies usually from the buttock area of representative psoriatic plaques immediately prior to treatment. Mitotic counts obtained from different plaques on different parts of the body taken at the same time in the same patient are usually equivalent (Fry & McMinn, 1970). Biopsy specimens were taken away from the plaque borders to provide the most representative mitotic counts since mitotic activity may be lower at the edge of a lesion (van Scott & Ekel, 1963; Porter & Shuster, 1967). Specimens were fixed in buffered formalin, processed for routine histopathological examination and stained with haematoxylin and eosin. The mitotic index of each patient's specimen was determined by counting all the mitotic figures in the epidermis in four non-adjacent histological sections of approximately 3 mm length (cut at 6 $\mu$m thickness), and dividing this number by the total length of the sections counted (mitotic index = number of mitoses per mm of linear surface). Post-treatment biopsies were taken, adjacent to the sites of the pretreatment biopsies, in the area of a psoriatic plaque, at the time of clearing.

Mitotic figures of prophase, metaphase, anaphase and telophase were recognized and counted according to the criteria of Scheving (1959). The cut-off stage for inclusion in prophase was an intact nuclear membrane, and in telophase was complete formation and separation of two distinct nuclear membranes. Our patients received treatment according to one of two investigational protocols. A sixteen centre study begun in July 1975 utilized a high intensity UV-A light unit developed by GTE Sylvania in conjunction with methoxsalen (8-methoxypsoralen) obtained from Elder Company. Results over an 18-month follow-up period have been published (Melski *et al.*, 1977). A second twelve centre clinical cooperative trial with methoxsalen from Westwood Pharmaceuticals Inc., was commenced in June 1975 utilizing mainly UV-A light units developed by General Electric and National Biological Corporation in addition to the GTE Sylvania system. Eighteen month follow-up results have been reported (Roenigk *et al.*, 1979). Treatment consisted of an oral dose of methoxsalen given according to body weight (approximately 0·65 mg/kg), followed in 2–3 h by exposure to high intensity UV-A recorded in J/cm$^2$. A major difference between the two protocols was the initial dose of light in joules which was higher in the earlier protocol (3·5 J/cm$^2$ vs. 1·5 J/cm$^2$ for skin type III). There were approximately equal numbers of patients in each group. Treatments were administered two to three times a week until there was 95% clearing of psoriasis, after which time maintenance treatments were given at less frequent intervals. The patients' clinical response was noted by a physician who was unaware of the mitotic count in each patient. The criteria used to assess this response were: (1) the number of treatments and joules administered to the time of initial response; (2) the number of treatments and joules needed until the patient was judged to be 95% clear of his lesions.

## RESULTS

Among seventy-five patients the mean total section length counted was 10·6 ± 1·2 mm. The mean mitotic index prior to treatment was 5·23 ± 1·2 (range 0·5–14). The mitotic index after treatment and resolution of previous psoriatic plaques in five patients was 0·3 s.d. ± 0·175. The mean standard deviation of the mitotic indices between each of the four sections in each patient was ± 1·2.

TABLE 1. Mean number of treatments to first response and to clearing for various mitotic indexes

| | | Mean no. of treatments | |
|---|---|---|---|
| Mitotic index | No. of patients | To first response | To clear |
| 0–1·9 | 12 | 7 | 22·3 |
| 2–3·9 | 25 | 3·8 | 30·1 |
| 4–5·9 | 20 | 4·8 | 24·0 |
| 6–7·9 | 13 | 7·3 | 24·5 |
| 8+ | 12 | 5·0 | 22·8 |

TABLE 2. Mean amount of UV-A to first response and to clearing for various mitotic indexes

| | | Mean UV-A (J/cm$^2$) | |
|---|---|---|---|
| Mitotic index | No. of patients | To first response | To clear |
| 0–1·9 | 12 | 6·0 | 229·8 |
| 2–3·9 | 25 | 3·3 | 243·2 |
| 4–5·9 | 20 | 3·1 | 180·4 |
| 6–7·9 | 13 | 4·7 | 210·6 |
| 8+ | 12 | 3·4 | 137·1 |

As can be seen from Tables 1 and 2 there was no correlation between the mitotic index and the number of treatments or the number of J/cm$^2$ until the time improvement was first noted. Nor was there a correlation between the mitotic index and the total number of treatments or the total number of joules for 95% clearing.

There were ten treatment failures. These were patients who did not show at least 95% clearing of their lesions in forty treatments. Pretreatment mitotic counts in these patients were evenly scattered throughout the whole range and had no predictive value as to response to PUVA treatment.

## DISCUSSION

It has been suggested that effective therapeutic agents which act on psoriatic lesions do so by direct inhibition of mitosis (van Scott & Reinertson, 1959; Weinstein & Frost, 1968). These authors and others have implied that there is a differential therapeutic effect upon rapidly growing epidermal cells. According to this hypothesis it would be expected that psoriatic plaques with a higher mitotic index, indicating a higher rate of proliferation of epidermal cells, would respond more quickly to PUVA treatment. In this study, the absence of a difference in the therapeutic response related to the rate of initial epidermal growth activity in psoriatic plaques suggests that the main therapeutic action of PUVA is by mechanisms other than a differential cytostatic effect related to the rate of proliferation of the epidermal cells.

A further argument (suggesting a mechanism other than a direct cytostatic effect of PUVA or of other therapeutic agents in psoriasis) is the observation that neither a treated psoriatic plaque nor normal skin develops the consequences of a mitotic rate that is below normal, i.e. atrophy or ulceration. Atrophy related to corticosteroid therapy occurs only after prolonged treatment, and not as an immediate sequel to involution of a psoriatic plaque.

Fry & McMinn (1968), studying the response of psoriatic plaques to dithranol and methotrexate, found that the first change in treated psoriatic plaques was the formation of a granular layer rather than a primary reduction in the mitotic count, suggesting a mechanism other than a direct cytostatic effect. This was not always the case after treatment with tar and corticosteroids. We have made similar observations.

The behaviour of treated psoriatic lesions is more suggestive of a correction of a metabolic defect, leading to a resumption of normal epidermal growth. Although there is some inhibition of mitosis by PUVA and antipsoriatic drugs, as has been shown in cell cultures (Walter *et al.*, 1973; Swanbeck & Liden, 1966), this alone does not suffice to explain the findings in treated patients.

## ACKNOWLEDGMENT

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# Methotrexate induced liver cirrhosis

## STUDIES INCLUDING SERIAL LIVER BIOPSIES DURING CONTINUED TREATMENT

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### SUMMARY

Seven hundred and sixty-four liver biopsies were performed in 328 psoriatics on treatment with methotrexate or being considered for systemic treatment either with methotrexate or with psoralens and long-wave ultraviolet light. The diagnosis of cirrhosis was established histologically in twenty-one patients. Two patients had cirrhosis in their premethotrexate biopsy and were not given methotrexate. The remainder all showed no signs of cirrhosis or fibrosis in their premethotrexate biopsy. The difference between the methotrexate treated psoriatics and the premethotrexate group was highly significant. Among thirty-nine patients treated for more than 5 years, ten developed cirrhosis (25·6%). Almost all patients were on a divided dose intermittent oral dosage schedule. The cumulative dose of methotrexate, when cirrhosis was first found, ranged from 590 to 8105 mg, with an average dosage of 2200 mg. Other factors contributing to cirrhosis in this study seem to be previous treatment with arsenic, a previous intake of alcohol, and lowered renal function.

Data on later serial biopsies from fourteen patients, of which eleven continued to receive methotrexate due to very severe psoriasis, seem to indicate that methotrexate induced liver cirrhosis is not of a very aggressive nature. When evaluated blind no progression was found in most of the later biopsies, and a 'cumulative cirrhosis index' composed of the combined gradings for fibrosis, assessment of membrana limitans, fibrous destruction and regeneration showed a tendency to decrease. In three patients the latest of the serial biopsies showed no cirrhosis. The observation period on continued methotrexate therapy ranged from 1 to 7 years. None of the patients with cirrhosis differed from the remaining patients on methotrexate in their laboratory results for evaluating liver damage, and apart from transient increases in serum glutamic pyruvic transaminases no abnormalities were found.

The data support the necessity of liver biopsies in the control of psoriatics treated with methotrexate. Liver biopsies should be performed at least in all psoriatics in whom a cumulative dosage of methotrexate exceeds 1·5 g. The data also indicate that methotrexate can be continued at least for a while in patients where the indication is strong enough, if the dosage is maintained as low as possible and alcohol consumption avoided.

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Isolated cases of cirrhosis of the liver in methotrexate treated psoriatics were reported beginning in 1968 (Coe, 1968; Epstein & Craft, 1969). Later cooperative (Weinstein *et al.*, 1973) as well as single studies (Dahl, Gregory & Scheuer, 1971; Roenigk *et al.*, 1971; Warin *et al.*, 1975; Zachariae, Grunnet & Søgaard, 1975; Nyfors, 1977) have confirmed that methotrexate may induce liver damage, which in some patients will lead to fibrosis or cirrhosis. Data from the international cooperative study indicated that increasing cumulative dosage, the combination of diabetes mellitus and obesity, increasing alcohol intake and increasing age were associated with liver damage in methotrexate treated patients. Long-term observations on methotrexate induced cirrhosis, however, have not been published and no studies have reported results on further liver biopsies taken from patients who continued to take methotrexate after the diagnosis of cirrhosis had been established. In some patients the severity of psoriasis may be such that continued treatment may be indicated in spite of the observed liver damage.

We have previously published results of liver biopsies in methotrexate treated psoriatics (Zachariae & Søgaard, 1973; Zachariae *et al.*, 1975). The purpose of the present paper is to report the increased frequency of cirrhosis appearing with increased cumulative dosage of methotrexate in our patients, as well as results of serial liver biopsies in patients with established methotrexate induced cirrhosis.

## MATERIALS AND METHODS

Our investigations were carried out on 764 liver biopsies from 328 psoriatics. The study comprises 240 premethotrexate biopsies and 461 postmethotrexate biopsies. One hundred and forty patients had pre- as well as postmethotrexate biopsies. The study also included sixty-three liver biopsies taken from psoriatics prior to treatment with psoralens and long-wave ultraviolet light (PUVA).

Methotrexate was originally administered intramuscularly in weekly dosages of 10–50 mg. From 1971 almost all patients have been on a divided dose intermittent oral dosage schedule over a 36 h period (Weinstein & Frost, 1969). Besides liver biopsies all patients have had blood tests at intervals of 1–4 weeks. The laboratory determinations for evaluating liver damage were serum glutamic pyruvic transaminase (SGPT), alkaline phosphatases, until 1973 bromsulphalein retention, and following 1973 a galactose tolerance test.

All biopsies were obtained by the Menghini technique (Menghini, 1958) using a 70 × 1·9 mm needle. Sections were cut 5 $\mu$m thick and stained with haematoxylin–eosin and van Gieson. Fatty infiltration, periportal inflammation, nuclear variability, focal necrosis, cholestasis, fibrosis, and cirrhosis were estimated. Except for cirrhosis each histological abnormality was graded as 1 (not present), 2 (slight), 3 (moderate) or 4 (severe). Biopsies where cirrhosis was established and all biopsies taken in patients subsequent to the diagnosis of cirrhosis being established were reinvestigated blind; membrana limitans, fibrous destruction, and regeneration were also graded. These gradings were added to the blind grading of fibrosis and together appear as grading of cirrhosis, when this was present.

In non-cirrhotic patients the intervals between serial biopsies were from 1 to 1½ years, in cirrhosis patients from 6 months to 1½ years. The longest observation period after cirrhosis was established was 7 years.

## RESULTS

The diagnosis of cirrhosis was established histologically in twenty-one patients. Two patients had cirrhosis in their premethotrexate biopsy and were not given methotrexate. The remainder showed no signs of cirrhosis or fibrosis in their premethotrexate biopsy, and had the diagnosis established later by serial biopsies taken during treatment. Table I shows the frequency of cirrhosis in patients prior to treatment compared with patients on treatment. The table shows the high frequency in

TABLE 1. Findings of cirrhosis in liver biopsies from psoriatics either prior to treatment or during treatment with methotrexate. Pretreatment biopsies include sixty-three liver biopsies from patients prior to PUVA treatment

| Groups | No. of patients | No. with cirrhosis | Frequency (%) |
|---|---|---|---|
| Pretreatment | 328 | 2 | 0·6 |
| Methotrexate treated | 183 | 19 | 10·3* |
| Patients treated > 2 years | 96 | 13 | 13·5* |
| Patients treated > 5 years | 39 | 10 | 25·6* |

For statistical evaluation a $\chi^2$ test was used.
* ($P < 0{\cdot}0001$).

patients treated with methotrexate for more than 5 years. The differences between the treated groups and the premethotrexate group were highly significant.

Three of our cirrhosis patients had previously had a heavy alcohol consumption with an intake of more than four drinks per day. However, they all had agreed to limit their intake to less than three drinks per week when put on methotrexate. Patients in general agreed to stop alcohol consumption when the diagnosis of cirrhosis was established. Seven patients had previously received arsenic elsewhere. The cumulative dose of methotrexate, when cirrhosis was first found, ranged from 590 to 8105 mg, with an average dosage of 2200 mg. Table 2 shows data on the eight patients with the lowest cumulative dosages.

Methotrexate was immediately discontinued in four of the patients when the diagnosis of cirrhosis was made. Two of these, however, later restarted on methotrexate due to a severe relapse of psoriasis which could not be controlled by other means including PUVA treatment. Five patients later discontinued methotrexate when their psoriasis was in a reasonable state. Twelve patients in all are still on continued methotrexate therapy, although several of these receive smaller doses than had been given previously.

Data on later serial biopsies are available from fourteen patients; eleven of these received continued methotrexate prior to their latest biopsy. The cumulative dosages appear in Table 5. Tables 3–5 show

TABLE 2. Data from eight patients developing cirrhosis prior to a cumulative methotrexate dosage of 1600 mg

| Patient no. | Cumulative dosage (mg methotrexate) | Comments |
|---|---|---|
| 5 | 590 | Lowered renal function |
| 2 | 810 | Previous arsenic treatment |
| 14 | 1000 | Previous arsenic treatment and high alcohol intake |
| 15 | 1025 | Child with generalized pustular psoriasis |
| 7 | 1120 | Generalized pustular psoriasis |
| 10 | 1270 | Previous arsenic treatment |
| 16 | 1465 | Previous arsenic treatment |
| 12 | 1585 | Previous arsenic treatment |

TABLE 3. Results of serial biopsies in cirrhosis patients. The findings are graded 1–4 (grade 1 is no pathological change). The average interval between each biopsy was approximately 1 year

| Patient No. | Sex | Age (years) | Fibrosis. Biopsy No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | Membrana limitans. Biopsy No. 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 62 | 2 | 2 | 3 | 3 | 2 | × | × | 2 | 2 | 3 | 3 | 2 | × | × |
| 2 | M | 67 | 2 | 2 | 2 | 2 | 2 | — | — | 1 | 2 | 2 | 3 | 2 | — | — |
| 3 | F | 44 | 3 | 2 | 2 | 2 | — | — | — | 3 | 2 | 2 | 2 | — | — | — |
| 4 | F | 27 | 2 | 2 | 2 | 2 | — | — | — | 3 | 2 | 1 | 1 | — | — | — |
| 5 | M | 73 | 2 | 2 | × | × | — | — | — | 2 | 1 | × | × | — | — | — |
| 6 | M | 54 | 1 | 2 | 2 | — | — | — | — | 2 | 1 | 1 | — | — | — | — |
| 7* | M | 63 | 2 | × | 2 | — | — | — | — | 2 | × | 1 | — | — | — | — |
| 8 | M | 67 | 3 | 3 | 4 | — | — | — | — | 2 | 3 | 3 | — | — | — | — |
| 9 | M | 68 | 2 | 3 | 2 | — | — | — | — | 2 | 3 | 2 | — | — | — | — |
| 10* | F | 65 | 3 | 3 | — | — | — | — | — | 3 | 3 | — | — | — | — | — |
| 11 | M | 74 | 2 | 2 | — | — | — | — | — | 1 | 2 | — | — | — | — | — |
| 12 | M | 66 | 2 | × | — | — | — | — | — | 1 | × | — | — | — | — | — |
| 13 | M | 59 | 2 | 2 | — | — | — | — | — | 2 | 2 | — | — | — | — | — |
| 14* | M | 60 | 2 | 2 | — | — | — | — | — | 3 | 1 | — | — | — | — | — |

(×) Cirrhosis not found. * Methotrexate was not given between first and last biopsy.
Average index first biopsy: 2·14 ± 0·53; 2·07 ± 0·73.
Average index last biopsy: 2·00 ± 0·67; 1·64 ± 0·77.

TABLE 4. Results of serial biopsies in cirrhosis patients. The findings are graded as in Table 2

| Patient No. | Sex | Age (years) | Fibrous destruction. Biopsy No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | Regeneration. Biopsy No. 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 62 | 1 | 1 | 3 | 2 | 2 | × | × | 1 | 1 | 2 | 2 | 2 | × | × |
| 2 | M | 67 | 1 | 1 | 1 | 2 | 2 | — | — | 1 | 2 | 2 | 1 | 1 | — | — |
| 3 | F | 44 | 2 | 1 | 1 | 2 | — | — | — | 2 | 2 | 2 | 2 | — | — | — |
| 4 | F | 27 | 2 | 1 | 2 | 1 | — | — | — | 2 | 2 | 1 | 1 | — | — | — |
| 5 | M | 73 | 2 | 1 | × | × | — | — | — | 2 | 1 | × | × | — | — | — |
| 6 | M | 54 | 1 | 1 | 1 | — | — | — | — | 1 | 1 | 1 | — | — | — | — |
| 7* | M | 63 | 1 | × | 1 | — | — | — | — | 2 | × | 2 | — | — | — | — |
| 8 | M | 67 | 3 | 3 | 4 | — | — | — | — | 2 | 2 | 3 | — | — | — | — |
| 9 | M | 68 | 2 | 2 | 2 | — | — | — | — | 2 | 2 | 2 | — | — | — | — |
| 10* | F | 65 | 2 | 2 | — | — | — | — | — | 2 | 2 | — | — | — | — | — |
| 11 | M | 74 | 1 | 2 | — | — | — | — | — | 1 | 1 | — | — | — | — | — |
| 12 | M | 66 | 1 | × | — | — | — | — | — | 1 | × | — | — | — | — | — |
| 13 | M | 59 | 1 | 1 | — | — | — | — | — | 2 | 1 | — | — | — | — | — |
| 14* | M | 60 | 2 | 1 | — | — | — | — | — | 2 | 1 | — | — | — | — | — |

(×) Cirrhosis not found. * Methotrexate was not given between first and last biopsy.
Average index first biopsy: 1·57 ± 0·65; 1·64 ± 0·50.
Average index last biopsy: 1·57 ± 0·92; 1·43 ± 0·70.

TABLE 5. Results of serial biopsies in cirrhosis patients. The findings are cumulative figures representing total scores from Tables 3 and 4 representing fibrosis, assessments of membrana limitans, fibrous destruction, and regeneration

| Patient No. | Sex | Age (years) | Cirrhosis Index or Biopsy No. | | | | | | | Cumulative methotrexate dosage (mg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Before cirrhosis | After cirrhosis |
| 1* | M | 62 | 6 | 6 | 11 | 10 | 8 | × | × | 1270 | 505 |
| 2* | M | 67 | 5 | 7 | 7 | 8 | 7 | — | — | 810 | 2340 |
| 3* | F | 44 | 10 | 7 | 7 | 8 | — | — | — | 3250 | 975 |
| 4 | F | 27 | 9 | 7 | 6 | 5 | — | — | — | 3710 | 1110 |
| 5 | M | 73 | 8 | 5 | × | × | — | — | — | 590 | 1670 |
| 6*† | M | 54 | 5 | 5 | 5 | — | — | — | — | 8105 | 1300 |
| 7 | M | 63 | 7 | × | 6 | — | — | — | — | 1120 | 0 |
| 8 | M | 67 | 10 | 11 | 14 | — | — | — | — | 2145 | 835 |
| 9† | M | 68 | 8 | 10 | 8 | — | — | — | — | 1680 | 350 |
| 10 | F | 65 | 10 | 10 | — | — | — | — | — | 1465 | 0 |
| 11 | M | 74 | 5 | 7 | — | — | — | — | — | 1720 | 630 |
| 12* | M | 66 | 5 | × | — | — | — | — | — | 1585 | 160 |
| 13* | M | 59 | 7 | 6 | — | — | — | — | — | 3290 | 1015 |
| 14*† | M | 60 | 9 | 5 | — | — | — | — | — | 1000 | 0 |

(×) Cirrhosis not found.
* Previously treated with arsenic; † previously high alcohol intake.
Average index first biopsy: 7·42 ± 1·99.
Average index last biopsy: 6·00 ± 0·96.

the results of the post-cirrhosis serial biopsies. In six biopsies from four patients the diagnosis of cirrhosis could not be confirmed in one or another later liver biopsy. In three patients the absence of cirrhosis was found in the latest biopsy taken. The tables also show that in all patients except one (Patient 8) there was a trend for later biopsies to be of the same rather low grading as found in the original biopsy, or even lower, irrespective of continued treatment with methotrexate. This tendency applies to all parameters investigated, but can most easily be found in the 'cumulative cirrhosis index' in Table 5.

None of our patients with cirrhosis differed from the remaining psoriatic patients on methotrexate in their laboratory results for evaluating liver damage. Disregarding transient increases in SGPT, no abnormalities were found.

## DISCUSSION

In our previous studies on liver biopsies in psoriatics the differences between pre- and postmethotrexate biopsies were never statistically significant (Zachariae & Søgaard, 1973; Zachariae *et al.*, 1975), although the latest published report showed that fibrosis and cirrhosis appeared in patients who had none of these signs in their premethotrexate biopsy. The data presented here clearly establish the increasing risk of developing cirrhosis with increasing cumulative methotrexate dosage.

As stated in the cooperative international study (Weinstein *et al.*, 1973) and by Nyfors (1977) the liver damage also may be related to other often multiple factors acting on the liver during methotrexate therapy such as alcohol intake, age, diabetes and obesity. Also the minor abnormalities often found in premethotrexate liver biopsies of psoriatics (Zachariae & Søgaard, 1973) may be a predisposing factor. Nyfors (1977) found a tendency for cirrhosis and fibrosis to develop more frequently in patients with pathological premethotrexate biopsies than in patients in whom these biopsies were normal. The cooperative international study showed that cirrhosis and fibrosis occurred more frequently in psoriatics receiving oral methotrexate in small daily doses than in those receiving larger weekly dosages. We have in an earlier study pointed out (Zachariae *et al.*, 1975) that previous intake of arsenic may also be a predisposing factor. As could be expected, our data show that special care should be taken to monitor liver damage in patients where for one reason or another methotrexate would be indicated and used in spite of lowered renal function.

On the other hand the results of our serial biopsies seem to indicate that methotrexate induced cirrhosis is not of a very aggressive nature, and that methotrexate can be continued at least for a while in such patients when the indication is strong enough. Such patients should of course have their dosage maintained at as low a level as possible, and alcohol consumption should be forbidden.

The improvement found in a number of our latest biopsies does not seem to be accidental. This could be due to better following of our recommendation concerning alcohol. Previous alcohol intake may very well have been higher in our patients than they admitted.

Our data support the necessity of liver biopsies in the control of psoriatics treated with methotrexate. Liver function tests alone are not a reliable indication of methotrexate induced hepatic fibrosis or cirrhosis. Liver biopsies should be performed in all psoriatics at least when a cumulative dosage of methotrexate exceeds 1·5 g.

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## ADDENDUM

Since this manuscript was submitted for publication Patient 1 (Tables 3–5) has had one further biopsy showing only slight fibrosis and no sign of cirrhosis.

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# Elastic fibres in the dermal papilla

## SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDIES

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### SUMMARY

The straight elastic fibres aligned perpendicularly to the dermo-epidermal junction have been examined by transmission and scanning electron microscopy. We found that these fibres connect the basal lamina to the underlying dermal elastic tissue, and it seems likely that they are involved in dermo–epidermal adhesion.

Thin elastic fibres perpendicular to the dermo–epidermal junction have been noted in the papillary dermis by several investigators (Dick, 1947; Hasegawa, 1960; Cooper, 1969; Cotta-Pereira, Rodrigo & Bittencourt-Sampaio, 1976). Hasegawa (1960) stated that they are oxytalan fibres because they can be demonstrated after oxidation with peracetic acid. By electron microscopy these oxytalan fibres appear as bundles of parallel fibrils (Cotta-Pereira *et al.*, 1976). These structures are similar to the fibrils surrounding true elastic fibres in the deeper dermis (Grenlee, Ross & Hartman, 1966; Hashimoto & Dibella, 1967; Bodley & Wood, 1972; Rodrigo, Cotta-Pereira & Riches, 1973) and have been termed 'elastic fibrils' (Kobayashi, 1968).

Several studies have shown the connection of the elastic fibrils to the basal lamina (Kobayashi, 1961, 1968; Briggaman & Wheeler, 1975; Cotta-Pereira *et al.*, 1976). Recently, Kobayashi (1977) demonstrated the actual connection by elastic fibrils from the basal lamina to a true elastic fibre.

The purpose of this paper is to demonstrate (i) the ultrastructure of these perpendicularly aligned elastic fibres, and (ii) the tridimensional architecture of the fibres by scanning electron microscopy.

### MATERIALS AND METHODS

Specimens were taken from the abdominal skin of normal subjects aged 25 and 38 years. These specimens were processed for scanning (SEM) and transmission (TEM) electron microscopy.

For SEM, a new technique was used (Tsuji, Lavker & Kligman, 1979). The specimens were incubated in 2 N sodium bromide at 37°C for 30 min, after which the epidermis was gently separated

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from the dermis. Approximately 1 mm thick transverse sections of the dermis were autoclaved in the water for 8 h at 121°C at 18 psi. The autoclaved specimens were fixed at room temperature in 2% paraformaldehyde–2·5% glutaraldehyde in 0·1 M cacodylate-HCl, buffered at pH 7·4 (Karnovsky, 1965) for 1 h, and then rinsed in buffer for 1 h after dehydration in a graded (70–100%) series of ethyl alcohol baths. The samples were then placed in 100% xylol for 2 h and air dried. Dried samples were mounted on aluminium stubs using silver paint. They were coated with a thin layer of gold palladium in an ISI sputter coater and viewed in a TV MINI-SEM operated at 10 kV.

For TEM, the specimens were fixed at 4°C for 1 h in 2% glutaraldehyde buffered with 0·2 M phosphate (pH 7·4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0·2 M phosphate (pH 7·4) for 1 h, and then dehydrated by graded ethyl alcohol and embedded in epon 812 according to the method of Luft (1961). Ultra-thin sections were cut with a Porter-Blum MT-2 ultramicrotome. They were stained on the grid for 1 h with a saturated aqueous solution of uranyl acetate and then for 10 min in Reynold's lead citrate. They were examined with a Hitachi HS 9 electron microscope.

## RESULTS

### *SEM findings*

The elastic fibres in the papillary dermis aligned perpendicularly to the skin surface displayed a smooth surface and were predominantly cylindrical in shape (0·5–2·5 μm in diameter). They were

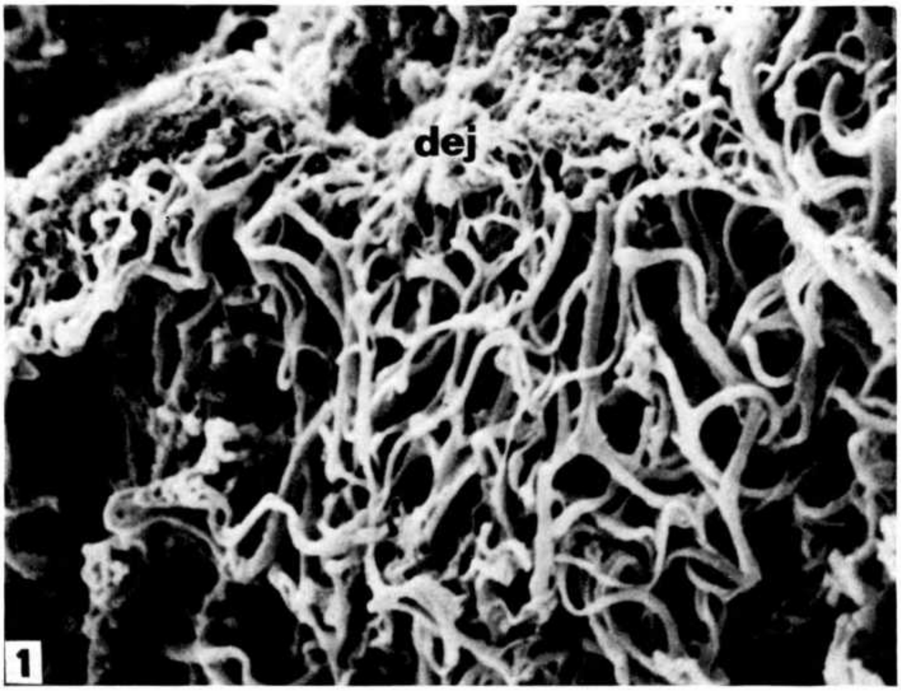


FIGURE 1. Scanning electron micrograph of a vertical section of the dermal papilla. The elastic fibres are aligned primarily perpendicularly to the surface, and they spray out to become fine fibres at the dermo–epidermal junction (dej). The fibres display a smooth surface and are predominantly cylindrical in shape (0·5–2·5 μm in diameter) (× 1080).

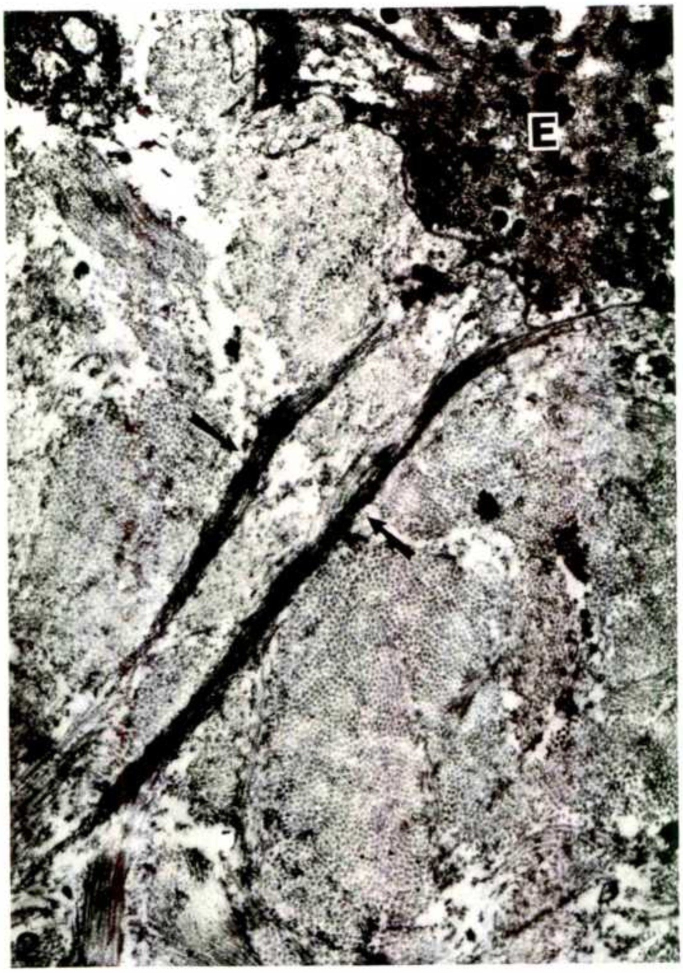
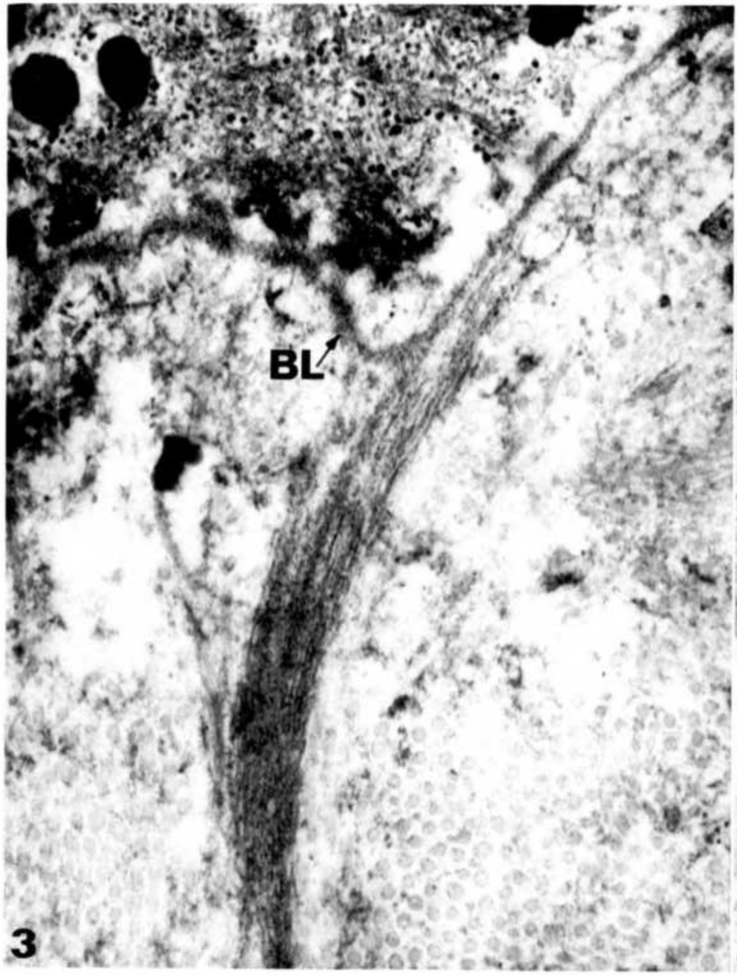


FIGURE 2. Transmission electron micrograph of the dermo–epidermal junction. Two long straight elastic fibres (arrowed) are seen to run perpendicularly from the deep to the superficial area without any interruption (E: epidermis) ( × 9600).

different from those in the lower parts of the dermis which were larger (1·5–2·5 $\mu$m in diameter) and elliptical (Tsuji *et al.*, 1979). Each of these fibres branched and sprayed into finer fibrils which were arranged in a vertical network (Fig. 1). All the fibres reached to the uppermost region of the dermis.

### *TEM findings*

Generally, the broad elastic fibres showed two kinds of components, an amorphous substance and a fibrillar component. In this study the fibrils will be called 'elastic fibrils'. In the dermal papilla, long (about 13 $\mu$m) and narrow (0·4 $\mu$m in diameter) straight elastic fibres were seen to run perpendicularly from the deep to the superficial area without interruption (Fig. 2). Superficially in the dermal papilla only elastic fibrils were observed (Fig. 3), but deep in the dermal papilla an amorphous material as well as the fibrils was seen (Fig. 4). No branches of the elastic fibril bundles were seen. One of the straight elastic fibres was seen to merge with the basal lamina (Fig. 3).



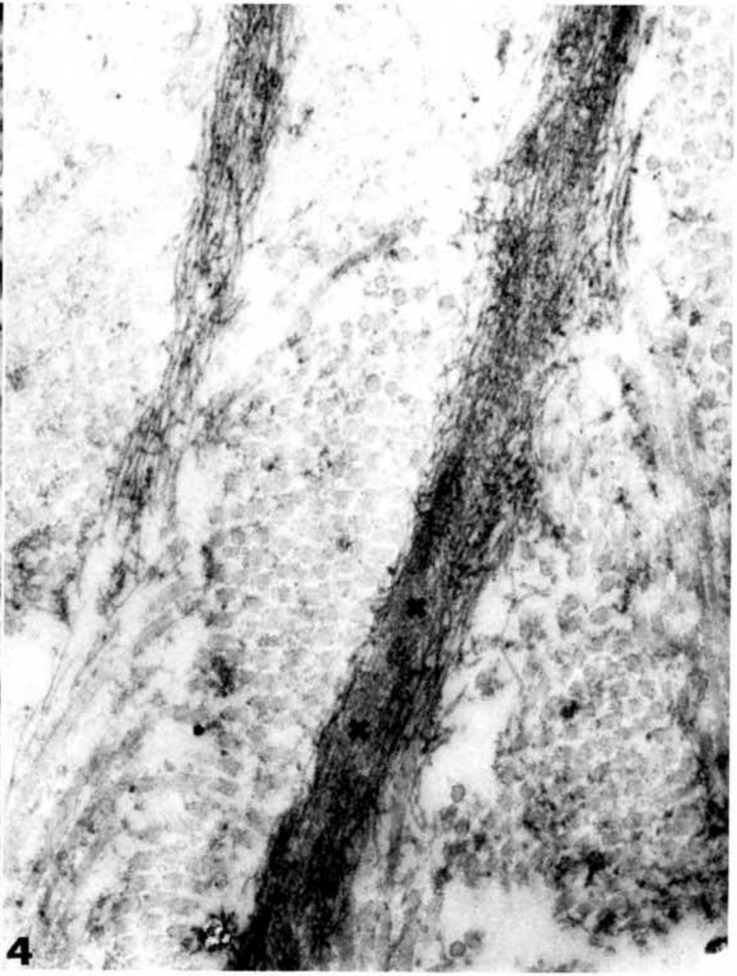


FIGURE 3. Higher magnification of the upper part of the larger elastic fibre of Fig. 2. A bundle of elastic fibrils is seen to merge with the basal lamina (BL) ( × 32,000).

FIGURE 4. Higher magnification of the lower part of the larger elastic fibre of Fig. 2. An amorphous substance (x) is seen inside the elastic fibril bundle ( × 32,000).

## DISCUSSION

By light microscopy, the oxytalan fibres (corresponding to the elastic fibril bundle) can be differentiated from the deeper elastic fibres by the staining methods used to characterize the elastic system fibres (Hasegawa, 1960; Cotta-Pereira *et al.*, 1976). On the other hand, our previous report (Tsuji *et al.*, 1979) showed that the fibres corresponding to oxytalan fibres remained after autoclaving just as the deeper elastic fibres did, while the other components of the dermis including collagen fibres were completely removed. In addition, the SEM findings of the oxytalan fibres showed the same appearance as those of the deeper elastic fibres except for the size, and they were continuous with each other (Tsuji *et al.*, 1979).

Several previous reports have demonstrated bundles of straight-arranged fibrils merging with the basal lamina, but they were too short for their connection with the dermal eleastic tissue system to be shown (Kobayashi, 1961, 1968; Briggaman & Wheeler, 1975; Cotta-Pereira *et al.*, 1976). The recent paper by Kobayashi (1977) showed the actual connection of elastic fibrils from the basal lamina to a true elastic fibre. However, the elastic fibrils which he showed to connect with the basal lamina were not from the main trunk but were a branch of the elastic fibril bundle continuous with the true elastic fibre.

The present paper has shown long straight elastic fibres, one of which was connected with the basal lamina. These findings were seldom seen, presumably due to the wavy shape of most of the elastic fibres, their varying, often considerable length, and their sporadic occurrence. The present study has confirmed that bundles of elastic fibrils are an extension of the dermal elastic tissue system, and suggests that the system is involved in anchorage of the epidermis to the dermis.

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# Antibodies to outer root sheath of hair

## DISTINCT ANTIGENIC PROPERTIES OF THE BASAL CELL LAYER OF THE OUTER ROOT SHEATH BELOW THE SEBACEOUS GLAND CANAL

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### SUMMARY

By screening 1500 sera obtained from patients undergoing a bone marrow graft, five sera have been selected which contained antibodies to the basal cell layer (B.C.L.) of the outer root sheath (O.R.S.) of hair follicles. It was demonstrated that these sera reacted with the B.C.L. of the O.R.S. but not with the B.C.L. of the adjacent epidermis. The change in antigenic properties was situated at the orifice of the sebaceous gland canal; thus basal cells below this orifice express an antigen distinct from those expressed by the epidermal basal cells. This is a novel example of tissue specific differentiation antigens that are linked to the type of keratinocyte differentiation.

It is now well established (Bystryn, Abel & Weidman, 1973; Bystryn, Nash & Robins, 1978; Saurat *et al.*, 1976) that some human sera contain antibodies to the cytoplasm of keratinocytes. Certain keratinocyte-cytoplasmic antibodies react only with the basal cell layer (B.C.L.) of stratified squamous epithelia (Bystryn *et al.*, 1978; Saurat, Didierjean & Gluckman, 1978a).

Several studies have suggested that the antigens to which keratinocyte-cytoplasmic antibodies are directed are differentiation antigens (Bystryn *et al.*, 1978; Saurat *et al.*, 1978a,b) or more precisely 'cell specific maturation antigens'.

The concept implies that within the skin, maturation is manifested by the gain or the loss of unique macromolecules as an individual cell differentiates. This is exemplified by basal cells which both lose and gain specific antigen as they migrate away from the dermo-epidermal junction (Bystryn *et al.*, 1978).

Even within the basal cell population, antigenic differences linked to differentiation have been demonstrated: basal cells of orthokeratinized substrates differ from those of parakeratinized substrates and mucosal substrates in their antigenic properties (Saurat *et al.*, 1978a, b). These findings have suggested that a basal cell, 'programmed' for orthokeratinization, would express in its cytoplasm

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molecules which differ antigenically from molecules expressed in the cytoplasm of a basal cell programmed for parakeratinization or mucosal differentiation. The type of maturation in the epithelium is therefore linked with the expression of distinct 'cell specific maturation antigens'.

The outer root sheath of hairs (O.R.S.) represents a distinct type of keratinocyte maturation as compared, for instance, to the adjacent orthokeratinized epidermis (Spearman, 1977). If the concept of cell specific maturation antigens is applied to the keratinocyte of O.R.S. these should differ antigenically from those of the adjacent epidermis. As far as the basal cells are concerned, no antigenic differences have been reported; the basal cells of O.R.S. were found to react identically to those of epidermis with a serum directed to B.C.L. (Bystryn *et al.*, 1978). In this paper, we demonstrate that the basal cells of O.R.S. (below the sebaceous gland duct) are antigenically different from those of orthokeratosis, parakeratosis and mucosa.

## MATERIALS AND METHODS

### *Sera*

Anticytoplasmic antibodies were identified by screening 1500 sera (dilution 1/10–1/50) from bone marrow graft recipients; these patients frequently produce epidermal cytoplasmic antibodies (Saurat *et al.*, 1976, 1977).

In this screening procedure, reactivity to cytoplasmic antigens was studied by routine indirect immunofluorescent staining on normal allogenic skin, rabbit lip and rabbit oesophagus. Seven sera from five subjects were selected, all having antibodies to B.C.L. in different titres (⩾1/100). In each of the sera, the antibodies to B.C.L. were found to be IgG antibodies.

The specificity for keratinocytes was demonstrated by the negative indirect immunofluorescence of the sera on the following substrates: liver, kidney, spleen, thyroid, trachea, aorta, gall bladder, pylorus, stomach, colon, duodenum, brain, adrenal glands, bone marrow, thymus.

These sera were thus considered as specific for keratinocytes (Saurat *et al.*, 1978b; Saurat, Didierjean & Galoppin, 1978c).

### *Substrates*

All the substrates were immediately frozen in liquid nitrogen after biopsy, and stored for no longer than 7 days at −30°C. The tissues were then embedded in O.C.T. compound (Tissue Teck, Ames Co. Indiana) and cut into 4 μm sections on a cryostat. Several substrates bearing keratinocytes involved in different types of maturation were used: (i) normal human skin from fifty subjects for orthokeratinization and hairs; (ii) rabbit lip specimens from thirty rabbits were cut sagitally in order to obtain a single specimen containing areas of orthokeratinization (with hairs), parakeratinization and of mucosa, and sharp junctional zones between these distinct types of differentiation, as previously reported (Saurat *et al.*, 1978a, b); (iii) mouse tail specimens (ten animals) which show areas of orthokeratosis (with hairs) and of parakeratosis with well defined junctional zones between the two types of maturation. All these substrates were cut perpendicular to the surface; (iv) human scalp punch biopsy specimens were obtained from ten subjects undergoing hair transplants. The specimens were cut both perpendicular to the surface and serially in a horizontal plane. Twenty horizontal serial sections, from the superficial epidermis to the hair bulbs, were studied for each specimen. This procedure facilitated a more precise analysis of the level at which the antigenic properties of O.R.S. change.

### *Conjugates*

Fluorescein labelled goat antihuman IgG conjugate was purchased from Hyland Co. (Costa Mesa, Calif.). It reacted specifically to human IgG by immunoelectrophoresis. The protein concentration was 15·2 mg/ml, the antibody content 2·0 mg/ml, the fluorescein isothiocyanate concentration 98·5 μg/ml and the fluorescein to protein molar ratio 2·7; the conjugate was diluted in PBS for 50 μg/ml antibody content.

### *Immunofluorescence tests*

Indirect immunofluorescence tests were performed by standard techniques as previously described (Saurat *et al.*, 1976). Slides were read by two observers with a Leitz orthoplan microscope with vertical illuminator for epi-illumination with an HBO 200 mercury vapour light and filters comb. I and S525 Leitz.

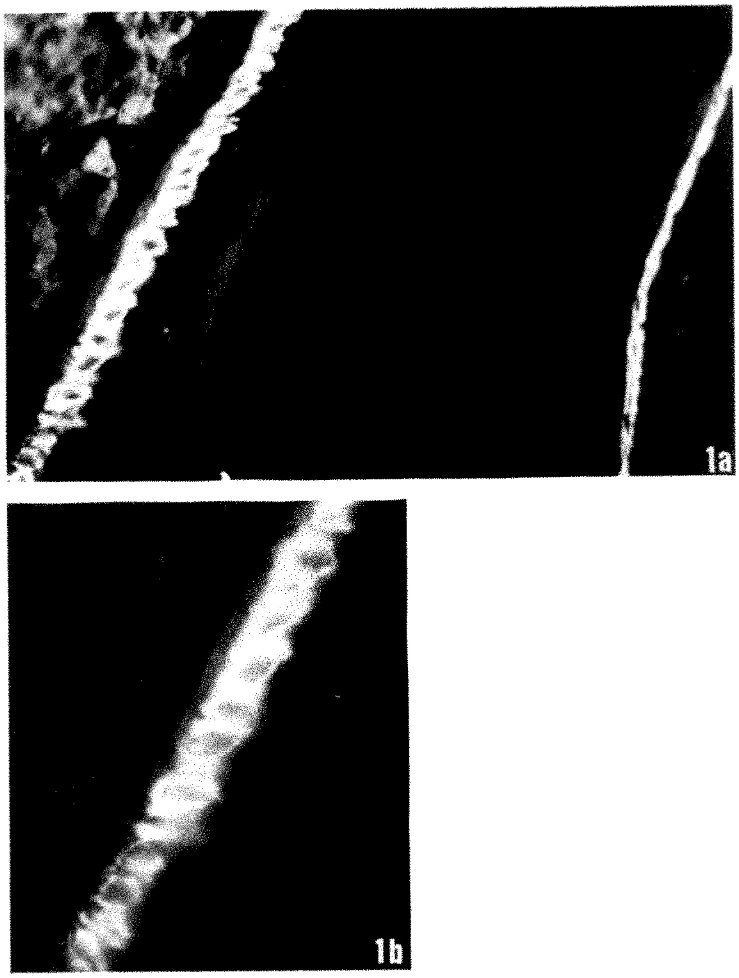

FIGURE 1. Appearance of the basal cell staining of outer root sheath of hairs. Indirect immunofluorescence with serum No. 634 (see Table 1) on rabbit lip specimen (Ektachrome 400 ASA, (a) ×200; (b) ×320).

## RESULTS

### *Staining of the B.C.L. of O.R.S.*

*Appearance of the staining.* Basal cell staining of O.R.S. was demonstrated with all seven sera. It was very similar to the staining of basal cells in epidermis (Fig. 1). The antigens were in the cytoplasm as evidenced by distinctly visible non-fluorescing intercellular spaces and nuclei. On vertical as well on horizontal section, the staining was localized within only one single layer of cells lining the dermo-epidermal junction. There was a sharp demarcation between this basal layer (stained) and the layer just above.

*Selective staining of O.R.S.* Staining of the B.C.L. of O.R.S. without any regular staining of the B.C.L. of adjacent epidermis (Fig. 2) was demonstrated with five sera (Table I). This was very reproducible, was observed with low (1/5) and high (1/200) dilution of the sera, and was unrelated to species: the same picture being observed on human skin, mouse tail and rabbit lip. Only sera Nos 570

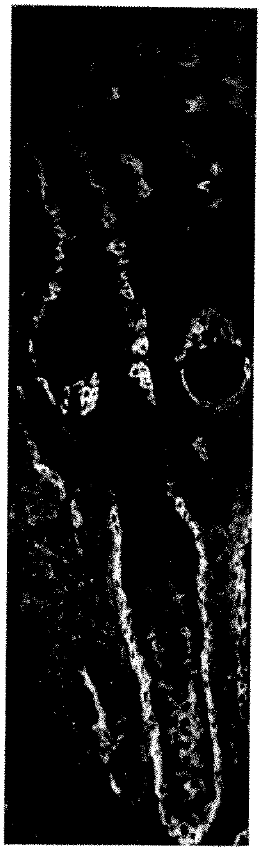

FIGURE 2. Basal cell staining of outer root sheath of hairs without any staining of adjacent epidermis. Indirect immunofluorescence with serum No. 634 (see Table 1) on rabbit lip specimen (Ektachrome 400 ASA, ×90).

TABLE 1. Results of indirect immunofluorescence on various substrates; rabbit lip (RL), human skin (H skin), human scalp (H scalp), and mouse tail epidermis (MTE). Sera Nos 28 and 1048 reacted (+ to + + +) with basal cells of epidermis and external root sheath of hairs. Sera Nos 569, 634, 118, 570 and 649 reacted with external root sheath of hairs but not with epidermis (–)

| | | Cytoplasmic staining* of basal cells of: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Epidermis | | | | External root of sheath of hairs | | | |
| Sera No. | Dilution | RL | H skin | H scalp | MTE | RL | H Skin | H Scalp | MTE |
| 28 | 1/5 | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 1/10 | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 1/50 | + + + | + + + | + + | + + | + + + | + + + | + + | + + |
| | 1/100 | + + | + + | + | + | + + | + + | + | + |
| | 1/200 | + | + | – | – | + | + | – | – |
| 1048 | 1/5 | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 1/10 | + + + | + + + | + + | + + | + + + | + + + | + + + | + + + |
| | 1/50 | + + | + + + | + | + | + + | + + + | + + | + |
| | 1/100 | + | + + | + | + | + | + + | + | + |
| | 1/200 | + | + | – | – | – | + | – | – |
| 569 | 1/5 | – | – | – | – | + + + | + + + | + + + | + + + |
| | 1/10 | – | – | – | – | + + + | + + + | + + | + + |
| | 1/50 | – | – | – | – | + + + | + + + | + | + |
| | 1/100 | – | – | – | – | + + | + + | + | + |
| | 1/200 | – | – | – | – | + | + | – | – |
| 634 | 1/5 | – | – | – | – | + + + | + + + | + + + | + + + |
| | 1/10 | – | – | – | – | + + + | + + + | + + | + + |
| | 1/50 | – | – | – | – | + + + | + + + | + | + |
| | 1/100 | – | – | – | – | + + | + + | + | + |
| | 1/200 | – | – | – | – | + | + | – | – |
| 118 | 1/5 | – | – | – | – | + + + | + + + | + + + | + + + |
| | 1/10 | – | – | – | – | + + | + + | + + | + + |
| | 1/50 | – | – | – | – | + | + | + | + |
| | 1/100 | – | – | – | – | + | + | – | – |
| 570 | 1/5 | – | – | – | – | – | + + + | + + + | + + + |
| | 1/10 | – | – | – | – | – | + + + | + + + | + + |
| | 1/50 | – | – | – | – | – | + + | + + | + |
| | 1/100 | – | – | – | – | – | + | + | – |
| 649 | 1/5 | – | – | – | – | – | + + + | + + + | + + + |
| | 1/10 | – | – | – | – | – | + + + | + + + | + + + |
| | 1/50 | – | – | – | – | – | + + + | + + + | + + |
| | 1/100 | – | – | – | – | – | + + | + + | + |
| | 1/200 | – | – | – | – | – | + | + | – |

* Number of experiments = 20–50.

and 649 did not react with rabbit lip. Finally the two sera (Nos 28 and 1048) which reacted with both O.R.S. and the adjacent epidermis were positive controls.

Such a selective staining of O.R.S. pointed to two problems: the upper and lower limits of this selective staining.

*Upper limit of the staining of O.R.S.* This was analysed on vertical sections on all the substrates and even more precisely on horizontal serial sections of human scalp. It was demonstrated that there

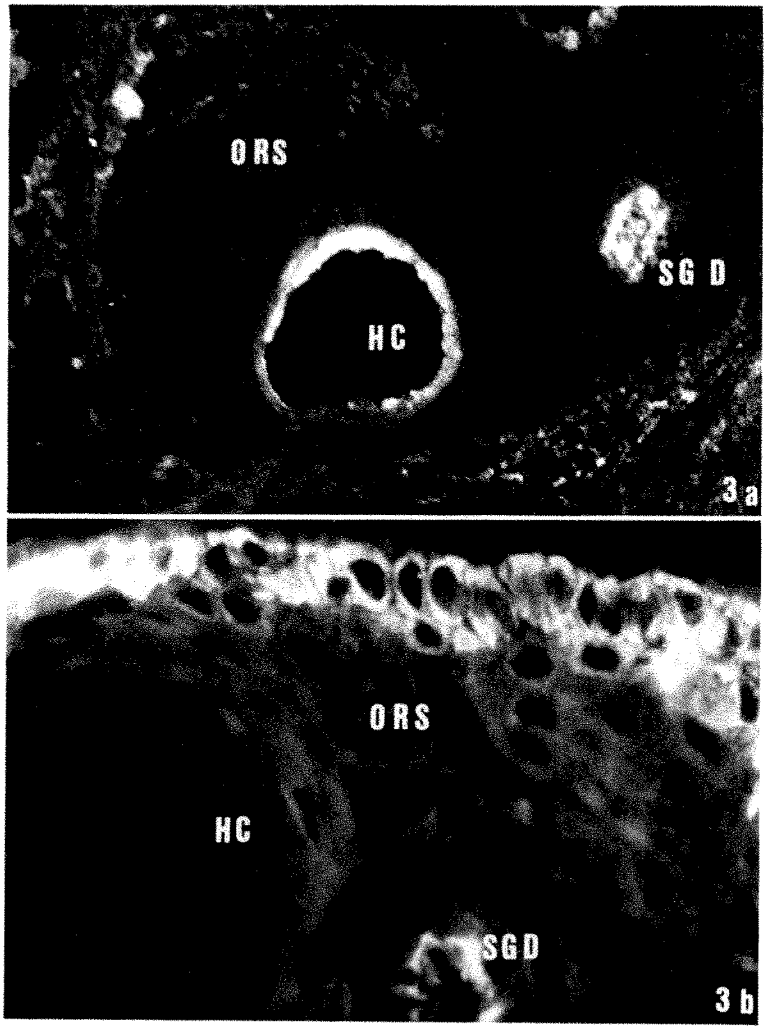

FIGURE 3. Horizontal section of human scalp at the level of the sebaceous gland duct. (SGD) sebaceous gland duct; (HC) hair canal; (ORS) outer root sheath. The fluorescence of the inner portion of SGD and HC is due to non-specific fluorescence. (a) Absence of staining of basal cells at this level with serum No. 649 (see Table 1); (b) staining at this level with serum No. 1048 which was found to react with basal cells in epidermis (Table 1).

was a sharp demarcation between stained and unstained basal cells. This demarcation was situated at the level where the sebaceous gland duct opens into the follicular canal (Figs 2 and 3).

*Lower limit of the staining of O.R.S.* This was difficult to analyse precisely: on vertical section it appeared that, when the section was parallel to the axis of the follicle, there was a zone at the lower part of the follicle which remained unstained. This zone corresponded with the lower part of the hair bulb.

The beginning of the staining was seen just around the hair bulb; at this level the stained cells had their axis parallel to the dermo-epidermal junction (Fig. 4). The horizontal serial sections correlated

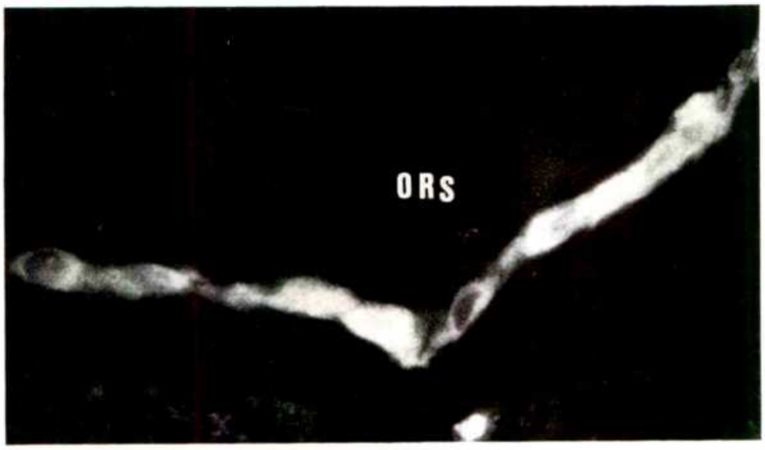

FIGURE 4. Horizontal section of human scalp. Lower portion of outer root sheath (ORS). This figure shows that the basal cells (stained by indirect immunofluorescence with serum No. 649 (see Fig. 3 and Table 1)) have their axis parallel to the dermal junction (Ektachrome 400 ASA, × 320).

well with these observations suggesting that only those cells of O.R.S. were stained, the cells in the matrix being unstained.

### *Staining of B.C.L. other than of the O.R.S.*

Table 2 shows that, even with some sera (Nos 569 to 649) showing only a selective staining of O.R.S. when orthokeratinized epithelia were used as substrates, there was in fact a uniform B.C.L. staining when parakeratinized or mucosal substrates were studied. Sera Nos 569 and 634 (Tables 1 and 2) appeared also to react with B.C.L. in parakeratinized and mucosal substrates, serum No. 118 with mucosal substrate. There were, however, two sera which reacted only with O.R.S. and not with the other types of keratinocyte maturation (Nos 570 and 649).

TABLE 2. This table shows that some sera giving a selective staining of outer root sheath of hairs (i.e. no staining of epidermis) contained antibodies to basal cells of parakeratinized and mucosal substrates. However, two sera (Nos 570 and 649) were specific only for outer root sheath (see Discussion)

| | I.I.F. cytoplasmic staining of basal cells on: | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Orthokeratinized substrates | | | Parakeratinized substrates | | Mucous substrates | | | External root sheath of hairs from: | | |
| Sera No. | Human skin | Rabbit lip | Mouse tail | Rabbit lip | Mouse tail | Rabbit oesophagus | Rabbit lip | Mouse lip | Human skin | Rabbit lip | Mouse tail |
| 28 | + | + | + | + | + | + | + | + | + | + | + |
| 1048 | + | + | + | + | + | + | + | + | + | + | + |
| 569 | – | – | – | + | + | + | + | + | + | + | + |
| 634 | – | – | – | + | + | + | + | + | + | + | + |
| 118 | – | – | – | – | – | + | + | + | + | + | + |
| 570 | – | – | – | – | – | – | – | – | + | – | + |
| 649 | – | – | – | – | – | – | – | – | + | – | + |

## DISCUSSION

These studies reveal the presence of an unique antigen in the B.C.L. of O.R.S. below the sebaceous gland duct. This has been demonstrated on several substrates from several species.

The identification of the unique antigenic properties has been made by using sera from bone marrow graft recipients; these patients have a disturbed immune system which might explain the production of (auto) antibodies with very narrow specificities (Grebe & Wayne-Streilen, 1976). By screening 1500 sera from fifty patients, we have found five sera that reacted to O.R.S. but not to adjacent epidermis. The fact that some of these sera also reacted with B.C.L. in other non-orthokeratinized stratified squamous epithelia may have two explanations: (i) there are common antigens for B.C.L. in different types of epithelia (traced by sera Nos 28 and 1048 in this study) and one distinct antigen in O.R.S.; (ii) each type of epithelium expresses a distinct antigen in the B.C.L., some sera containing a mixture of antibodies to several of them.

The latter explanation is more concordant with our results shown in Table 2.

The distinct antigenic property of B.C.L. of O.R.S. is expressed from the lower zone of O.R.S. (with flattened cell parallel to the dermo–epidermal junction) up to the sebaceous gland canal. The O.R.S. is known to be divided into two regions (Spearman, 1977): (i) the region around the follicular canal which is considered an extension of the surface epidermis since its biochemical, functional and electron microscopic characteristics are similar to the epidermis and different from the lower zone of the O.R.S. In this study, the zone around the follicular canal was found to have antigenic properties similar to the epidermis; (ii) the zone below the sebaceous gland canal, considered as the true follicular outer root sheath. The cells in that zone differ from those of the follicular canal in several features (Spearman, 1977).

Therefore the keratinocytes in O.R.S. below the sebaceous gland duct show a distinct type of maturation. It was found in this study that B.C.L. in that zone expressed a distinct antigenic specificity.

This is another argument for the concept of cell specific maturation antigen (Bystryn *et al.*, 1978): differentiation antigens are commonly thought of in terms of tissue specific antigens; the process of differentiation from embryonic cells to several tissues in a single individual leads to antigenic differences between cells of different organs in this individual. However, within a single organ, differentiation continues and might be associated with the loss or the gain of unique macromolecules. Stratified epithelia built of keratinocytes are examples of distinct types of differentiation, i.e. orthokeratinization, parakeratinization, mucosal differentiation; we have shown (Saurat *et al.*, 1978a, b) that each of these types of differentiation is associated with the expression of distinct antigenic properties in the cytoplasm of keratinocytes.

This also seems to be the case for O.R.S. below the sebaceous gland canal which express a 'specific maturation antigen'.

The tracing of such subtle changes in antigenic properties has been made 'by chance' with sera produced in an experimental situation in man. It is hoped that the chemical identification of the target antigens will be achieved and may serve for the understanding of the keratinocyte differentiation process.

## ACKNOWLEDGMENTS

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# The dysgonic form of *Microsporum canis* in N.W. London

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## SUMMARY

An account is given of the occurrence in N.W. London of ringworm due to the dysgonic form of *Microsporum canis* in cats, dogs, and humans. The unusual morphology of the dysgonic strain is described as is the extraordinary lability of the fungus in culture. The distribution of cases in man and animals in the area is mapped. The clinical and epidemiological behaviour of the fungus, as illustrated by four family outbreaks, differs little from that of the typical strain. The origin of the dysgonic strain is unknown.

Since atypical strains of *Microsporum canis* were first described by Sabouraud in 1910 they have been mentioned by comparatively few workers. Duncan (1945, 1948) and Walker (1950a, b) both referred to 'glabrous' varieties in their surveys of the dermatophytoses of Great Britain and recently English (1978) and English & Tucker (1978) have described some atypical and dysgonic strains isolated in N.W. London.

A brief account is given here of some of the epidemiological and clinical features of the cases in N.W. London.

## DESCRIPTION OF THE DYSGONIC STRAIN

The dysgonic strain has been described in detail by English (1978) and its main characteristics only will be described here. Briefly, young colonies are pale in colour, thick and raised at the centre, with a markedly feathery periphery of mainly submerged mycelium (Fig. 1). A brilliant yellow pigment is produced at some time during the first 2 weeks, but fades rapidly. By about 6 weeks colonies become very thick and deeply cerebriform (Fig. 2), khaki to pinkish in colour, and have a suede-like surface.

The mycelium is very distinctive (Fig. 3). Hyphae are thick with closely spaced, very short, lateral branches, many of them growing in the opposite direction to the main hypha (reflex branching). This 'dysgonic mycelium' gives rise at intervals to tufts of apparently normal mycelium which in turn gradually revert to the dysgonic form, giving a cyclical alternation between the two forms and

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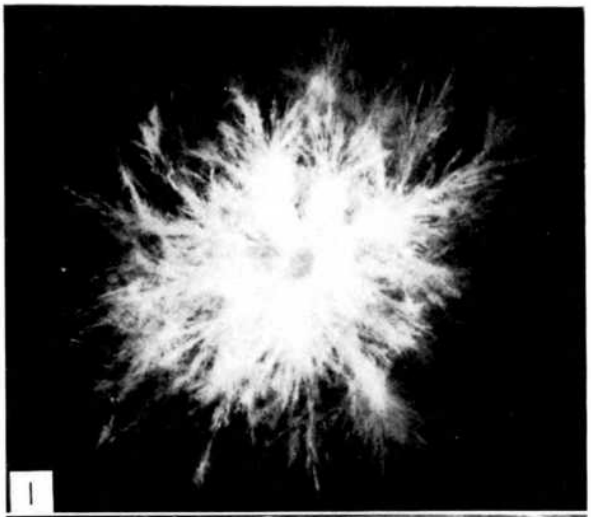

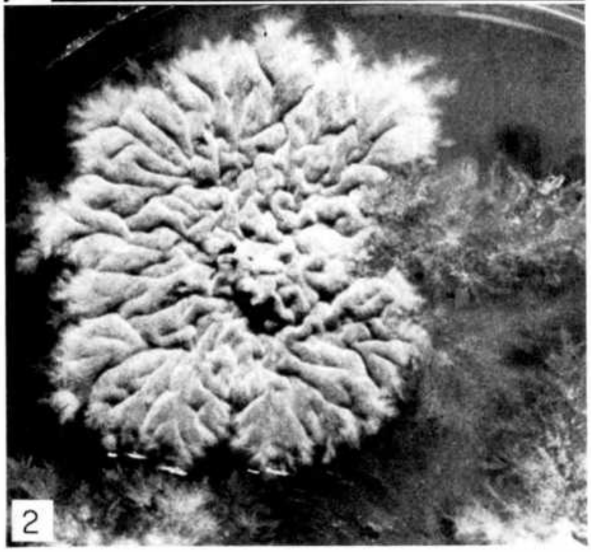

FIGURE 1. Dysgonic colony of *M. canis* at 2 weeks, showing feathery submerged mycelium.
FIGURE 2. Dysgonic colony of *M. canis* at 6 weeks, with cerebriform thickening.

accounting for the tufted appearance of the feathery edges of young colonies. Neither macroconidia nor microconidia are produced by the fungus in the dysgonic state.

The dysgonic strain is very unstable. The majority of colonies, long before reaching the cerebriform stage, are swamped by sectors of fast-growing mycelium (Fig. 4) some of which are reversions to the typical *Microsporum canis* strain, and others a bewildering array of 'atypical' strains differing from one another in colour, growth rate, and mycelial type. Subculture of the original dysgonic strain at this stage is often impossible and this may account for reports of atypical strains isolated directly from the living host. Macroconidia and microconidia are produced by all atypical strains (English & Tucker, 1978) but macroconidia are often misshapen, sometimes to a bizarre degree (Fig. 5). Experimental infection of guinea-pigs with dysgonic strains has shown that fluorescence of hairs occurs similar to

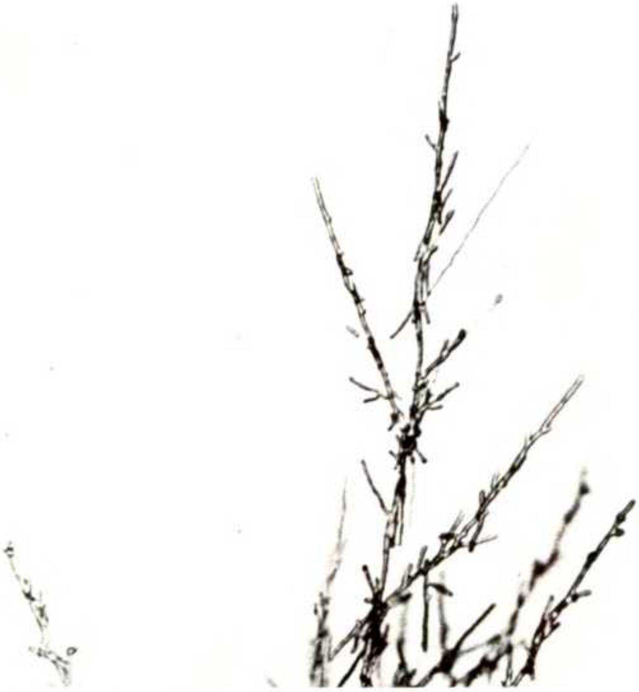

FIGURE 3. Dysgonic mycelium at 7 days, showing thickened hyphae with reflex branching (approx × 200).

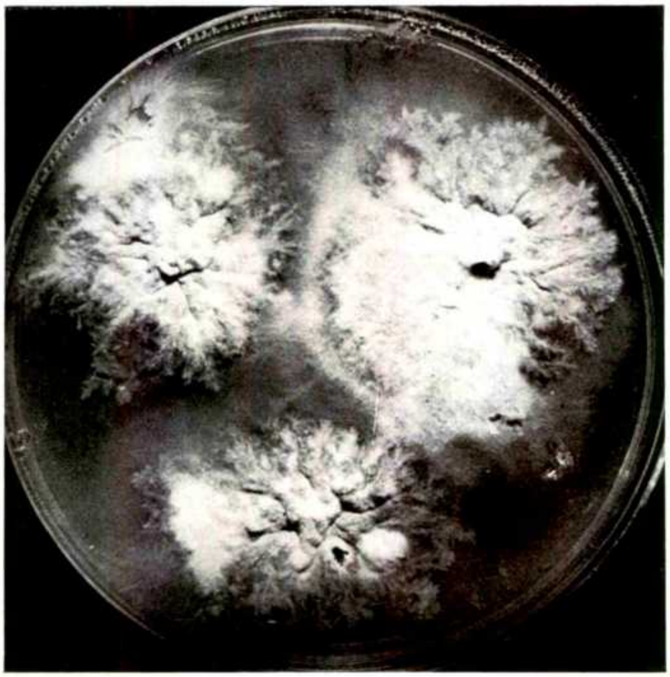

FIGURE 4. Dysgonic colony of *M. canis* at 6 weeks, demonstrating overgrowth with rapidly developing mutants.

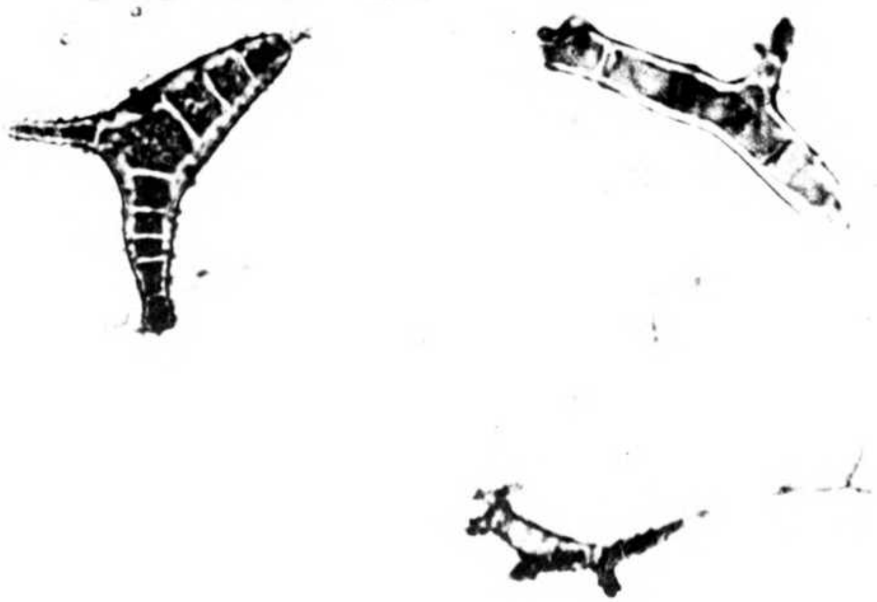

FIGURE 5. Misshapen macroconidia of atypical strains of *M. canis* (approx × 800).

that seen in infections with typical strains (English, Gentles & Ball, 1979). Fluorescence has also been demonstrated in ten of thirteen natural human infections with dysgonic strains (Gentles, personal communication).

## ORIGIN OF CASES

The human and animal cases reported here were those from which specimens had been sent to the laboratory for diagnostic purposes by local veterinary and general practitioners. The survey covers the years 1974–1978; tracing of contacts commenced in 1976. It seems probable that other cases occurring in the vicinity may not have come to our attention, either because treatment was provided on a clinical diagnosis only, or because specimens have been examined in mycological centres elsewhere.

## OCCURRENCE OF INFECTION

Details of the isolations of the typical and dysgonic strains of *M. canis* made from man and animals between 1974 and 1978 are shown in Table 1 and the location of these cases is shown in Fig. 6.

The dysgonic strain was first seen in our laboratory in October 1974. Examination of laboratory records failed to reveal an isolation during the previous 2 years and the first case, Mrs M.'s cat, presented initially as an isolated incident. The following year, however, there were three further isolations, all of human origin. The possibility of a reservoir of this fungus existing in the district was then considered and an attempt made to assess the size of the problem. This deliberate search for new cases, rather than a true increase in the incidence of the disease, may account for the increasing isolation rate.

TABLE 1. Dysgonic and typical forms of *Microsporum canis* isolated in NW London between 1974 and 1978

| Year | Host | No. of cases<br>Typical strain | No. of cases<br>Dysgonic strain |
|---|---|---|---|
| 1974 | Feline | | 1 |
| 1975 | Human | | 3 |
| 1976 | Human | | 2 |
| | Feline | | 1 |
| 1977 | Human | | 2 |
| | Feline | 2 | 6 |
| | Canine | 2 | |
| 1978 | Human | 1 | 9 |
| | Feline | 1 | 12 |
| | Canine | | 1 |

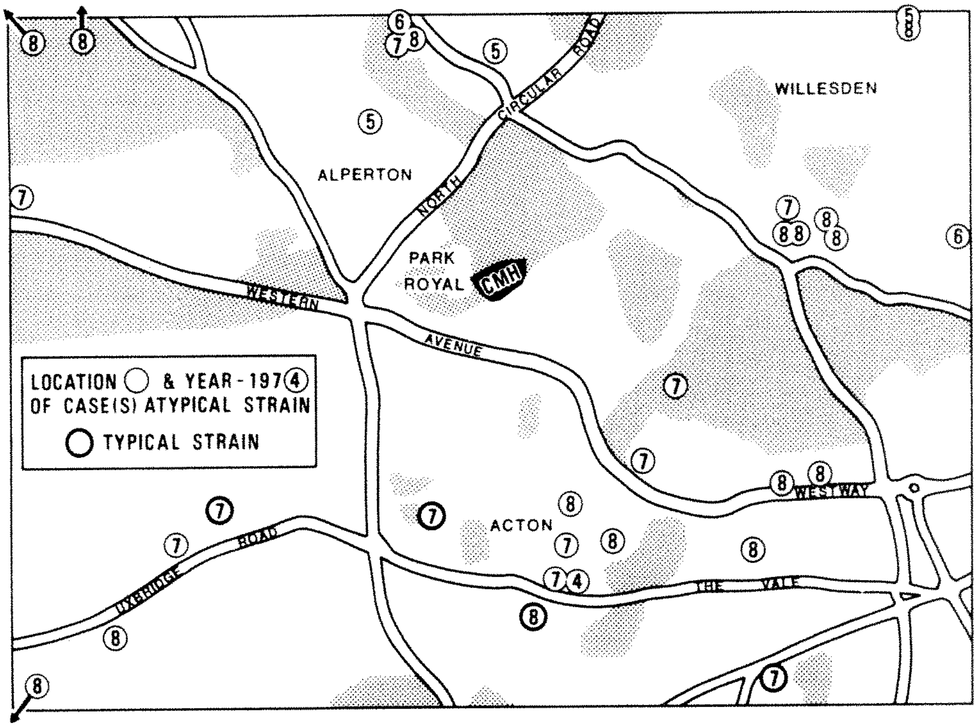

FIGURE 6. Map of the section of N.W. London surrounding Central Middlesex Hospital (C.M.H.). The shaded areas denote non-residential localities, including parks, factories, and railway sidings. The circles differentiate between typical and dysgonic strains, but not between human and animal.

## CASE HISTORIES

*Mrs M.'s cat* (1974)

Mrs M. suffered from what clinically appeared to be tinea corporis (material was not examined mycologically); this responded to topical antifungal treatment. Her cat, however, exhibited hair loss concurrently with her own infection. Scrapings of skin and fur from the cat were obtained. These revealed hyphae on direct microscopy and a dysgonic strain of *M. canis* was isolated.

*Mrs B.* (1976)

A dysgonic strain of *M. canis* was isolated from a lesion on the wrist of Mrs B. When later the household pets (cat, kitten, dog, and rabbit) were examined, only the kitten had a visible lesion. This was a granulating mass on the tail at the site of an old injury; a heavy growth of *M. canis* (dysgonic) was isolated from this site. Although the dog and cats had the free run of the house, including the bedroom, there was no evidence that Mr B had been infected.

*The family D.* (1977)

The first case seen in this family was a child (D.D.) aged 12, with lesions on the chest and back; scrapings of these showed fungal hyphae on direct microscopy, and yielded *M. canis* (dysgonic) on culture. Several weeks later the child's mother developed a ringworm lesion on the lower leg, and the father had a similar lesion on his left shoulder; it was noted that the family kept a cat. When first seen Mrs D.'s lesion had regressed to a flat circle of scaly skin, with vesicles barely visible; but that of Mr D. was erythematous, with marked vesiculation, particularly at the raised periphery. The cat had a granulating lesion on the left ear, which was believed to have been caused by fighting. Direct microscopy of material from patients and cat revealed fungal elements and *M. canis* (dysgonic) was isolated in each case.

*Mrs Q.* (1978)

This lady visited her general practitioner because of a ringworm lesion on the face. Hyphae were seen on direct microscopy and dysgonic *M. canis* was later isolated. Two out of three household cats yielded dysgonic *M. canis* on culture. One had a granulating lesion on the ear that yielded a heavy growth of the fungus; the other had no visible lesions, but yielded a very light growth of dysgonic *M. canis* from fur brushings. These two animals lived out of doors in a shed; the third cat, however, which was kept indoors was not shown to be infected.

In both human and animal infections the clinical features caused by typical and dysgonic strains are indistinguishable.

## DISCUSSION

The dysgonic form of *Microsporum canis* is unique among dermatophytes not only in its peculiar morphology, but also in the contrast between its apparent stability in the host and its extreme lability in culture. The outbreak of infection in N.W. London has continued for at least 5 years, during which time sixteen human and twenty-one animal cases have been identified, while only six isolations of the typical strain have been obtained.

Family infections with the dysgonic strain occurred, and undoubtedly represent host to host transmission. With regard to the feline infections, most cats were within easy prowling distance of another case, and it is probable that these animals are the main vectors of the fungus. Despite numerous passages the dysgonic strain, so unstable in culture, has remained stable as long as it grows on the living host. But after a few weeks in culture fast-growing typical and atypical sectors eventually overwhelm the host-stable dysgonic form.

The source of the typical isolates obtained in the present study is unknown. It has been shown (English & Tucker, 1978) that it is possible for the typical form to arise as a sector of the dysgonic strain *in vitro*; this however is unlikely to be the case *in vivo*. Atypical strains such as arise from the dysgonic strain have not been obtained on primary culture from the N.W. London outbreak, although it has been previously demonstrated that they readily arise from subcultures. English *et al.* (1979) have shown that these forms, like the typical and dysgonic strains, are pathogenic for guinea-pigs.

Information from colleagues indicates that the dysgonic strain may be found in small foci in several parts of the country, but data on the extent and duration of outbreaks, or on whether the dysgonic strain is eventually replaced by the apparently more stable typical strain, are not available. Continued study of the N.W. London outbreak may provide this information.

## ACKNOWLEDGMENTS

I wish to express my sincere thanks to Dr Mary P.English for her great help and encouragement which have made this paper possible. Also, I would like to thank Drs P.G.Brent and S.McKeigue, and the veterinarian Mr T.T.Farrell, for their continued interest and for information on and access to their patients. In addition, I would like to thank Dr D.A.McSwiggan for helpful criticism, Mr G.James, Department of Medical Illustration, Bristol University, for drawing the map, and Mr J.M.Ethrington, Photographic Department, Central Middlesex Hospital.

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*British Journal of Dermatology* (1980) **102,** 437.

# Facial follicular porphyrin fluorescence: correlation with age and density of *Propionibacterium acnes*

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## SUMMARY

The incidence and distribution of facial follicular porphyrin fluorescence was studied in a large group of subjects ranging in age from 1 to 90 years. Fluorescence appears first on the nose and chin, increases in incidence through adulthood and declines after the age of 50 possibly reflecting the rate of sebum secretion. We have shown that the intensity of fluorescence is proportional to the *P. acnes* population and confirmed that coproporphyrin III is produced *in vivo*.

As early as 1927 it was recognized that punctate orange-red fluorescence could be visualized when the face is examined under ultraviolet light (Bornmer, 1927). The source of this fluorescence was unknown until Cornelius & Ludwig (1967) demonstrated that a strain of *Propionibacterium acnes* produced coproporphyrin III, accounting for the orange–red fluorescence seen under Wood's light. More recently Lee *et al.* (1978) examined several strains of *P. acnes* and *P. granulosum* and showed that both species synthesized coproporphyrin III.

The use of cutaneous porphyrin fluorescence has been advocated as a simple, non-invasive technique for monitoring the effectiveness of systemic antibiotics. Martin *et al.* (1973) showed that successful reduction of *P. acnes* is accompanied by a reduction in porphyrin fluorescence, a valuable correlation which allows rapid evaluation of patients who show a poor clinical response to systemic antibiotics. We undertook this study in order to determine the number of *P. acnes* required to produce follicular fluorescence, the prevalence of this phenomenon, the age of onset, and whether any patterns of onset or intensity of fluorescence exist.

## MATERIALS AND METHOD

### *Subjects*

A total of 193 males and 185 females, varying in age from 1 to 90 years, were examined in a dark room with Wood's light. The presence of follicular orange–red fluorescence on the nose, forehead, cheek,

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and chin was recorded. Subjects were asked to avoid the use of any perfume, soap, or cosmetic on the day of examination in order to avoid the possibility of quenching of fluorescence. Twenty subjects were examined weekly for a period of 3 months. No significant variation in the degree or intensity of follicular fluorescence was seen, except in occasional subjects who forgot to avoid the use of facial cosmetics. Subsequent subjects were examined on one occasion only.

### *Correlation of P. acnes and intensity of fluorescence*

We examined 112 males aged from 18 to 35 years under Wood's light, and the intensity of fluorescence on the forehead was rated as negative, weak, moderate, or intense. Each subject was then quantitatively cultured by the detergent scrub technique of Williamson & Kligman (1965). This method involves placing a sterile glass cylinder with an internal area of 3·8 $cm^2$ over the area to be sampled, adding 1 ml of 0·1% Triton X-100 in 0·075 M phosphate buffer, scrubbing with a blunted Teflon spatula for 1 min, and then withdrawing this sample fluid. This procedure is repeated and the two samples are pooled and then diluted in 10-fold steps in 0·05% Triton X-100. A 0·025 ml volume of each dilution was placed on brain heart infusion agar (Baltimore Biological Lab.), supplemented with 0·8% dextrose, 0·1% Tween 80, 0·5% yeast extract, 1% sodium lactate, and 0·5% of a salt solution containing 4 g of $MgSO_4 \cdot 7H_2O$, 0·4 g of $MnSO_4 \cdot 4H_2O$, 0·4 g of $FeSO_4 \cdot 7H_2O$ acidified with two drops of 10 N $H_2SO_4$ (BH1A+). Plates were incubated for 7 days at 37°C in a Gas Pak anaerobic system. *P. acnes* was identified by colonial morphology, susceptibility to *P. acnes* bacteriophage (ATCC 29399B), and standard biochemical tests (McGinley, Webster & Leyden, 1978). Repeated cultures were not deemed necessary since we have previously demonstrated that non-significant variation is seen when subjects are sampled on multiple occasions (Leyden *et al.*, 1975).

### *Characterization of porphyrin*

Follicular material was obtained by expressing the contents of large nasal follicles of two males with intense fluorescence. This material consists mainly of desquamated follicular cells, sebum, and *P. acnes*. The expressed material was extracted with 3 ml of ethyl acetate for 10 min, centrifuged to remove debris, and then evaporated to dryness. The sample was redissolved in 0·5 ml of ethyl acetate and 20 $\mu$l was spotted on Whatman #1 chromatography paper. Standards of coproporphyrin III (Sigma) and protoporphyrin (Sigma) were also spotted. Chromatograms were developed in 2,6-lutidine: water (5:3·5 vol/vol) in an ammoniacal atmosphere and then visualized under Wood's light (Falk, 1964).

## RESULTS

### *Correlation of P. acnes and intensity of fluorescence*

Porphyrin fluorescence intensity was proportional to *P. acnes* density (Fig. 1). The geometric mean density of *P. acnes* in those with no fluorescence was 88 organisms/$cm^2$, compared to 8746 for those with weak fluorescence, 269,898/$cm^2$ in those with moderate, and 1,223,207/$cm^2$ in those with intense fluorescence. The geometric mean for each group was significantly different from each other (Student's unpaired '*t*'-test).

### *Survey of porphyrin fluorescence*

*Age differences.* There were obvious differences in follicular fluorescence in different age groups (Table 1). Few (3–4%) children under 5 years of age demonstrated fluorescence. In the 6–10 year age range there was a slight increase (11–17%) in fluorescence. The 11–15 age group showed a marked

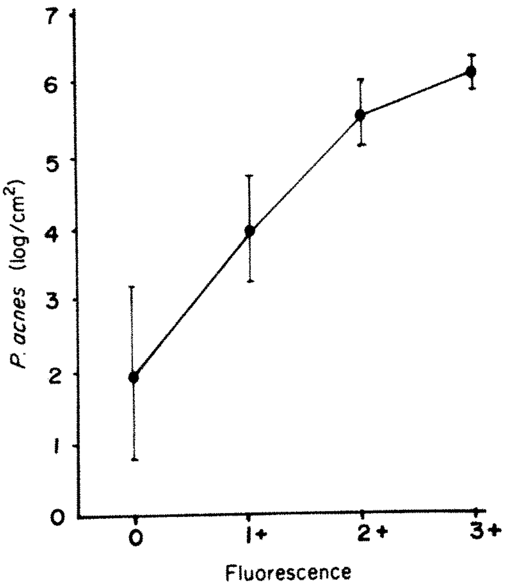

FIGURE 1. Follicular porphyrin fluorescence.

increase in the percentage of those with fluorescence, which was further increased in the 16–20 year range. From 20 to 50 years there were few differences in incidence. In the 51 – 70 year group, and particularly in those over 70, there was a fall-off in the incidence of fluorescence (Table 1).

*Site differences.* The first sites to show fluorescence were the nose and the chin in children (Table 1). In adults, the forehead and cheek had an incidence of fluorescence similar to that found on the nose

TABLE 1. Follicular porphyrin fluorescence (% incidence)

| Age (years) | 1–5 | 6–10 | 11–15 | 16–20 | 21–30 | 31–50 | 51–70 | 71–90 |
|---|---|---|---|---|---|---|---|---|
| Male | 39 | 19 | 28 | 27 | 36 | 26 | 14 | 14 |
| Forehead | 3 | 0 | 14 | 81 | 72 | 38 | 36 | 29 |
| Cheek | 5 | 0 | 14 | 63 | 50 | 56 | 36 | 14 |
| Nose | 3 | 16 | 57 | 85 | 81 | 88 | 57 | 36 |
| Chin | 3 | 11 | 36 | 93 | 86 | 56 | 36 | 14 |
| Female | 53 | 18 | 29 | 18 | 20 | 17 | 15 | 15 |
| Forehead | 0 | 0 | 17 | 39 | 55 | 18 | 13 | 7 |
| Cheek | 0 | 0 | 24 | 39 | 30 | 18 | 20 | 13 |
| Nose | 4 | 17 | 59 | 61 | 75 | 59 | 40 | 20 |
| Chin | 4 | 11 | 48 | 61 | 65 | 47 | 33 | 20 |
| All subjects | 92 | 37 | 57 | 45 | 56 | 33 | 29 | 29 |
| Forehead | 1 | 0 | 16 | 64 | 66 | 27 | 24 | 17 |
| Cheek | 2 | 0 | 19 | 53 | 43 | 24 | 28 | 14 |
| Nose | 3 | 16 | 58 | 76 | 79 | 73 | 48 | 28 |
| Chin | 3 | 11 | 42 | 80 | 79 | 73 | 34 | 17 |

and chin. In the older age groups (51–70 and 71–90) there was a trend for the incidence of follicular fluorescence to decrease most rapidly on the forehead.

*Sex differences.* No significant differences were seen with respect to the incidence of fluorescence in children or young adults. In women above 30, however, there was a significantly lower incidence.

*Porphyrin analysis.* Paper chromatography of the follicular contents extract detected a compound with an Rf identical to that of the coproporphyrin III standard. No protoporphyrin was detected.

## DISCUSSION

In this study we have shown that: (1) the intensity of porphyrin fluorescence correlates with the density of *P. acnes* populations; (2) the incidence of porphyrin fluorescence increases from childhood to adulthood and then decreases in those over 50; (3) follicular porphyrin fluorescence appears first on the nose and chin; (4) the prevalence of porphyrin fluorescence is greater in males over 30 than in females in this age group; and (5) we have confirmed earlier reports that show the orange–red fluorescence to be due to coproporphyrin III.

The intensity of porphyrin fluorescence was directly related to the density of *P. acnes*. As shown in Fig. 1, those with either weak or no fluorescence had wider ranges of *P. acnes* than those with moderate or heavy fluorescence. The results suggest that a density of *P. acnes* in excess of 1000 organisms (log 3·0) is required before definite follicular fluorescence is discernible.

The increase in porphyrin fluorescence with increasing age from childhood to teenage and young adulthood parallels our previous findings of increasing densities of *P. acnes* populations with age (Leyden *et al.*, 1975). Likewise, the increased incidence of fluorescence in males over 30 also correlates with our findings of increased levels of *P. acnes* in males in those age groups. Similarly, the finding that porphyrin fluorescence decreases in the elderly correlates with our findings of lower levels of *P. acnes* in that age group (Leyden *et al.*, 1975). Sebum appears to be a major factor in determining the size of *P. acnes* populations, as both *P. acnes* density and porphyrin fluorescence parallel age-related differences reported for sebum production (Pochi & Strauss, 1974; Strauss & Pochi, 1968; Cunliffe & Shuster, 1969).

The pattern of development of fluorescence is interesting. The nose and chin were clearly the first areas to develop follicular fluorescence. Since porphyrin fluorescence appears to require in excess of $10^3$ *P. acnes* per $cm^2$, one would predict that children under the age of 10 should have very low *P. acnes* populations on their face, since follicular fluorescence is uncommon in that age group. This, in fact, has been shown in two separate studies (Matta, 1974; Leyden *et al.*, 1975). Since the nose and chin develop fluorescence first, we would expect that sebaceous gland development, and subsequent *P. acnes* population, would occur in those areas prior to development on the forehead and cheek. In this regard, it is of interest that acne vulgaris, in which there is sebaceous gland hyperactivity and increased densities of *P. acnes*, also frequently occurs first on the nose.

Since facial follicular fluorescence correlates well with *P. acnes* levels we have found the non-invasive technique of examination under Wood's light to be useful in monitoring antibiotic therapy in acne vulgaris. As previously shown by Martin *et al.* (1973), this is an extremely useful technique in evaluating patients who do not respond to systemic antibiotics. With topical agents, this technique is somewhat limited by the possibility of quenching, i.e. absorption of porphyrin fluorescence by a compound in the topical agent, masking the fluorescence of high densities of *P. acnes*.

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## *Pharmacology and Treatment*

# A double-blind trial of metronidazole versus oxytetracycline therapy for rosacea

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## SUMMARY

Forty patients with papulo-pustular rosacea were treated for 12 weeks on a random double-blind basis either with oxytetracycline 250 mg twice daily or with metronidazole 200 mg twice daily. Both drugs produced an improvement which was greater after 12 weeks than after 6 weeks, but there was no significant difference between them. Metronidazole appears to be a safe and effective drug for the treatment of rosacea.

The antibiotic metronidazole (Flagyl) has been shown to be significantly better than a placebo in the treatment of rosacea (Pye & Burton, 1976) but it has not been tested against conventional therapy; we have now performed a double-blind trial of metronidazole versus oxytetracycline.

## MATERIALS AND METHODS

Forty patients with papulo-pustular rosacea were treated either with oxytetracycline 250 mg twice daily (30 min before meals) or with metronidazole 200 mg twice daily on a random double-blind basis, coded tablets being issued by the pharmacist. None of the patients had received antibiotics for 4 weeks prior to the trial. Each patient was assessed before treatment by one author (E.S.), the severity of the papulo-pustules being graded as mild, moderate or severe. The patients were assessed again after 6 weeks' and 12 weeks' treatment and the patients' condition relative to the initial visit was independently recorded as 'worse', 'unchanged', 'slightly improved', 'moderately improved' or 'much improved', by the same doctor and by the patient. The patients were photographed in colour under standard conditions at each visit and after their final visit the three photographs were evaluated by the second author (J.L.B.). The two photographs taken during therapy were compared with the initial photographs and the changes noted were recorded in the same way. The independent opinions of the patient and two doctors were thus obtained at 6 and 12 weeks for each subject and a numerical value was assigned to each of the three opinions, as follows: worse (−1); unchanged (0); slightly improved (1); moderately improved (2); much improved (3). The mean of the three opinions was then recorded and only then was the treatment code broken for the further analysis of the results.

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TABLE 1. Degree of improvement in rosacea, expressed on an arbitrary numerical scale (mean ± s.d.) described in the text

| | Oxytetracycline | Metronidazole |
|---|---|---|
| 6 weeks | 1·8 ± 0·9 | 1·6 ± 0·9 |
| 12 weeks | 2·6 ± 0·7 | 2·3 ± 1·0 |

## RESULTS

Twenty patients receiving oxytetracycline and eighteen patients receiving metronidazole completed the trial. The results (Table 1) show that both drugs produced an improvement, and there was no significant difference between them. Both drugs produced significantly more improvement after 12 weeks than after 6 weeks ($P < 0{\cdot}05$). No side-effects were reported in either group, but two patients taking metronidazole failed to attend for follow-up after the initial visit.

## DISCUSSION

Several groups have shown that metronidazole is an effective treatment for rosacea (Pye & Burton, 1976; von Braun, Koester & Taube, 1978; Guilhou *et al.*, 1978) and the present double-blind study has shown that the response to metronidazole does not differ significantly from that produced by oxytetracycline.

The beneficial effect of metronidazole in rosacea cannot be explained since the cause of the disease is unknown. Metronidazole acts against a wide variety of protozoa and anaerobic bacteria, but it also has anti-inflammatory and immunosuppressive effects (Tanga, Antami & Kabade, 1975; Grove, Mahmoud & Warren, 1977).

Metronidazole is a relatively safe drug, although it can occasionally cause alcohol-induced headaches by a disulfiram-like effect (Penick, Carrier & Sheddon, 1969) and neuropathy has also been reported (Coxon & Pellis, 1976). It has mutagenic effects in some bacteria (Voogd, van der Stell & Jacobs, 1974) but the significance of this in man is uncertain.

Oxytetracycline is probably a safer drug than metronidazole for long-term use but the latter has the advantage that it is tolerated well during pregnancy and has no adverse effects on the offspring (May & Baker, 1979) whereas tetracycline administered in the last trimester causes discoloration of the fetal teeth.

We conclude that metronidazole is a worthwhile drug for the treatment of papulo-pustular rosacea, especially in patients who have not responded to tetracyclines, or for pregnant women in whom systemic treatment is essential.

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# The guinea-pig: an animal model for human skin absorption of hydrocortisone, testosterone and benzoic acid?

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## SUMMARY

$^{14}C$ ring-labelled hydrocortisone, testosterone and benzoic acid dissolved in acetone were applied to the backs of guinea-pigs (4 $\mu g/cm^2$). Percutaneous absorption was quantified by following the excretion of tracer in urine and faeces for 5 days. Absorption of hydrocortisone and benzoic acid was 2·4% (s.d. = 0·5; n = 3) and 31·4% (s.d. = 9·1; n = 3) of the applied dose respectively, similar to published human absorption data. Testosterone was absorbed to a greater extent in guinea-pigs (34·9% ± 5·4; n = 5) than man.

A thioglycollate based depilatory cream significantly increased the skin absorption of testosterone, while the absorption velocity was unaltered.

Two analytical methods were compared, direct counting versus wet ashing; results were in the same range for the three compounds. Two methods of quantifying skin absorption were compared; urine recovery corrected for incomplete urinary excretion after parenteral administration versus the addition of urine and faecal recovery. For benzoic acid, excreted mainly in the urine (88·1%), the two methods gave similar results; for testosterone, excreted in the urine to a lesser extent (46·8%), the method of addition of urine and faecal recovery appeared to be more correct.

Although interspecies variation may limit the value of animal models, new animal models yielding results similar to those obtained in man are continuously sought. This report describes the use of the guinea-pig as a model for human skin absorption. Three substances (hydrocortisone, testosterone, and benzoic acid) were used representing low, middle and high penetrants of human skin. We compare two ways of pre-application skin treatment, shaving versus shaving plus chemical depilation and two different analytical methods.

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## MATERIALS AND METHODS

### *Animals*

Three to six adult Hartley strain guinea-pigs of both sexes were housed individually in metabolic cages with urine/faeces separators and collectors and unlimited access to water and guinea-pig pellets. Lettuce and carrots given daily increased urinary output.

### *Radioactive compounds*

Benzoic acid, testosterone and hydrocortisone ring-labelled with radioactive carbon ($^{14}C$) (New England Nuclear) were used. The radiochemical dose was a maximum 1 $\mu$Ci per guinea-pig.

### *Application techniques*

To correct the skin absorption data for incomplete urinary excretion, parenteral control experiments were performed for each compound, using subcutaneous injections and later intraperitoneal injections (Feldman & Maibach, 1969). In a few subcutaneous injections, leakage from the injection site was noticed within a few hours; subsequently this application method was abandoned.

For topical application, the compounds were applied in acetone solution at 4 $\mu$g/cm$^2$ as used by Feldmann & Maibach (1970) in human experiments. This allowed comparison between guinea-pig values and human values taken from the literature (Feldmann & Maibach, 1969, 1970). The compounds were applied to the shaved back with a 50 $\mu$l Hamilton syringe to a 1·75 cm$^2$ circular area demarcated by indentations in the skin made by pressing a ring (diameter 1·5 cm) against the surface for 30 s. The acetone was evaporated during application by blowing and was visible for less than 15 s.

The application site was separated from the remaining surface by a glass cylinder (outer diameter 3 cm, inner diameter 2 cm, height 1 cm) glued to the shaved skin with cyanoacrylate glue (Krazy glue). The cylinder top was covered with non-occlusive tape preventing the chemical, hair and stratum corneum from contaminating the metabolic cage, urine and faeces. After 24 h, the glass cylinder was removed; the remaining test material was wiped away with soaked cotton swabs and the application site dried with one dry cotton swab. The glass cylinder, tape and cotton swabs were placed in cups with 50 ml of demineralized water until counting.

For testosterone we evaluated the effect of chemical depilation on skin absorption using a thioglycollate cream hair remover. The cream was applied to the skin for 6 min leaving no visible irritation. The tracer application took place immediately after depilation.

### *Analysis*

Urine was collected for 5 days with the first divided into four periods (0–4, 4–8, 8–12 and 12–24 h). After each time period, the collector was washed in demineralized water (20–40 ml) to remove the tracer remaining in the collector. The wash water was added to the urine sample. After weighing and aliquoting, the urine was refrigerated until counting. Faeces were collected daily and blended with 200 ml of demineralized water and refrigerated until counting. The $^{14}C$ content of 2 ml aliquots was measured by two different methods:

(1) Direct counting with appropriate internal standards in a Toluene-Triton-PPO (2,5-diphenyloxazole) scintillation solution (2 l toluene, 1 l triton X-100 and 15 g PPO). A Beckman 200B liquid scintillation counter was used and counts per minute recorded. The counting efficiency varied from 36 to 80% depending upon the urine concentration. The samples were counted with and without internal standard.

(2) The other method involved wet ashing of the urine and trapping the carbon as $CO_2$ in organic base. The 'burning' solution was concentrated sulphuric acid with 50 g potassium dichromate and

50 g chromium trioxide added per 5 l. As organic absorbing base Carbosorb (Packard) was used. This method is described in detail (Maibach & Feldmann, 1967). After wet ashing, the organic base was dissolved in a toluene-PPO scintillation solution (20 g PPO/l toluene) with 0·5–1·0 ml methanol added to each counting vial as a clearing agent. The counting efficiency was about 85%.

Only the wet ashing method was used with faeces. Results were calculated as per cent of the applied dose for each urine collection using a pre-programmed Monroe computer. From this value, the absorption rate (%/h) and total absorption per 5 days were calculated. The skin absorption values were corrected for incomplete urinary excretion using the correction factor obtained from the parenteral studies (Feldmann & Maibach, 1969). An alternative method involved addition of urine and faeces values.

The time excretion rate relationship was plotted on semilogarithmic curves and the linear regression line calculated for each experiment.

TABLE 1. $^{14}C$ recovery in urine after parenteral administration (wet ashing method)

| Material | % Recovery ± s.d. | Half-life (h) | Excretion correction factor | No. of animals |
|---|---|---|---|---|
| Hydrocortisone | 66·4 ± 9·8 | 10·3 | 1·50 | 3 |
| Testosterone | 46·8 ± 4·5 | 14·1 | 2·13 | 3 |
| Benzoic acid | 88·1 ± 4·3 | 9·5 | 1·13 | 4 |

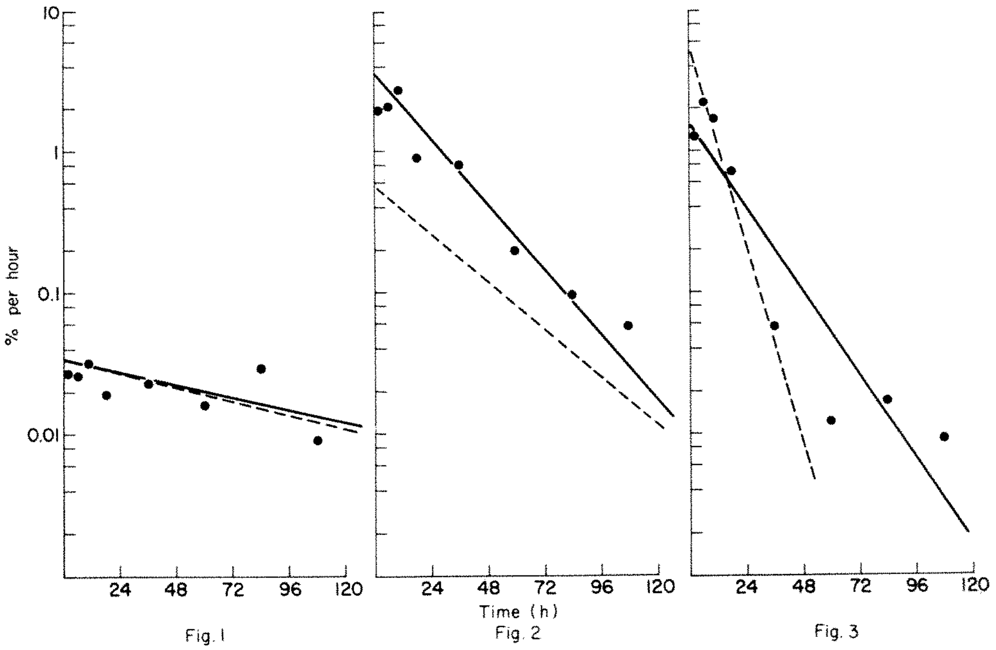

Fig. 1 Fig. 2 Fig. 3

FIGURES 1–3. Semilogarithmic plots and regression lines of guinea-pig excretion rates for hydrocortisone (Fig. 1), testosterone (Fig. 2) and benzoic acid (Fig. 3) compared to human data from Feldmann & Maibach (1969, 1970). (Solid line) guinea-pig; (broken line) human.

TABLE 2. $^{14}C$ recovery in urine after cutaneous application (wet ashing method). Results corrected for incomplete urinary excretion

| Material | Excretion rate (%/h) 0–4 | 4–8 | 8–12 | 12–24 | 24–48 | 48–72 | 72–96 | 96–120 | % Total | s.d. | $r^2$* | No. of animals | Half-life (h) | Human values |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hydrocortisone | 0·03 | 0·03 | 0·02 | 0·02 | 0·02 | 0·02 | 0·03 | 0·01 | 2·41 | 0·53 | 0·38 | 3 | 93·6 | 1·87 ± 1·59 |
| Testosterone | 0·69 | 1·41 | 1·55 | 1·25 | 0·64 | 0·18 | 0·06 | 0·03 | 50·73 | 12·30 | 1·00 | 5 | 16·6 | 13·24 ± 3·04 |
| Benzoic acid | 1·28 | 2·19 | 1·68 | 0·71 | 0·06 | 0·01 | 0·02 | 0·01 | 31·41 | 9·10 | 0·83 | 3 | 12·3 | 42·62 ± 16·45 |

* $r^2$ = coefficient of determination.

TABLE 3. Guinea-pig skin absorption: comparison of analytical methods, effect of depilation, and recovery from application site 24 h after application

| | Percent of dose absorbed (±s.d.) | | | | | |
|---|---|---|---|---|---|---|
| | Urine recovery (corrected for incomplete excretion) | | Recovery from urine plus faeces | | | Recovery from application site after 24 h. |
| | | | | Wet ashing | | |
| Penetrant | Wet ashing | Direct counting | Wet ashing | +depilation | −depilation | Direct counting |
| Hydrocortisone | 2·4±0·5<br>($r^2$ = 0·38)† | 2·9±0·3<br>($r^2$ = 0·13)† | | | | |
| Testosterone | 50·7±12·3<br>($r^2$ = 1·0)† | 49·2±11·3<br>($r^2$ = 0·96)† | 34·9±5·4 | *<br>45·3±4·6<br>(half-life 16·7 h) | *<br>34·9±5·4<br>(half-life 16·6 h) | 34·8±7·7<br>16·0±1·6<br>14·7±1·6 |
| Benzoic acid | 31·4±9·1<br>($r^2$ = 0·83)† | 31·2±9·7<br>($r^2$ = 0·88)† | 28·9±8·9 | | | |

* Mann Whitney U test: two tailed $P = 0·036$.
† $r^2$ = coefficient of determination.

## RESULTS

The $^{14}C$ recovery in urine after parenteral administration of the test materials shows that biological half-lives after parenteral administration are considerably less than the half-lives for topical administration (compare values in Tables 1 and 2), thus allowing the use of urinary excretion as a measure of percutaneous absorption. The percutaneous absorption experimental data (Table 2) are corrected for incomplete urinary excretion using the parenteral excretion data.

Figures 1–3 present the results graphically with the comparable data regarding human skin absorption taken from Feldmann & Maibach (1969, 1970). As in the human studies, there was a considerable variation in absorption.

The total absorption of testosterone was greater than that for benzoic acid; in man the opposite was the case. Using the alternative method for quantifying skin absorption, namely, addition of recovery found in urine *and* faeces, the total absorption for testosterone amounts to 34·9±5·4% (Table 3). Thus the guinea-pig skin absorption of testosterone is doubled compared to the human values using both methods of calculation. Benzoic acid was absorbed less in guinea-pigs compared to humans; there was better agreement between the two methods of quantifying the skin absorption for benzoic acid (Table 3). The absorption of hydrocortisone in guinea-pigs is similar to that of man regarding absorption rate and total absorption.

The comparison of the two analytical methods is shown in Table 3. The standard deviation ranged from 10% to 31% of the mean suggesting a considerable interindividual variation in skin absorption.

The influence of chemical depilation just prior to application is shown in Table 3. The difference between shaving plus depilation for 6 min and shaving without subsequent chemical depilation was significant. The total absorption was increased by chemical depilation, while the velocity of absorption remained unchanged.

The amount of chemical remaining on the application site and on the glass ring with tape varied from experiment to experiment—even with the same sampling technique (Table 3).

## DISCUSSION

The guinea-pig skin absorption data for hydrocortisone and benzoic acid resemble the reported human values regarding absorption rate and total absorption, while about three times more testosterone was absorbed from guinea-pig skin than from human skin (Feldman & Maibach, 1969). The high values for testosterone absorption in the guinea-pigs, obtained by correcting for incomplete urinary excretion, might be false due to low recovery of testosterone in urine after parenteral administration. Steroids are metabolized and transformed in human skin (Berliner, 1969) making the urinary excretion of metabolites of questionable value regarding skin absorption. The method of correcting for incomplete urinary excretion using the correction factor obtained from parenteral experiments assumes that urinary excretion constitutes the major metabolic pathway. Summation of recoveries of testosterone from urine (corrected for incomplete excretion), from a wash of the application site obtained after 24 h and from washing the glass ring and tape, exceeded 99% in one animal. This indicates that the correction factor method might overestimate the testosterone absorption, because residual tracer deposits in the skin and appendages after 5 days would probably raise recovery above 100%. For the testosterone experiments, the alternative method of adding urinary and faeces excretion together seems more correct. For benzoic acid, excreted mainly through the kidneys (88%), the correction factor method is more appropriate. Only minor differences were found in Table 3 when comparing the two methods for benzoic acid. For hydrocortisone, the skin absorption was low as in man. The coefficient of determination ($r^2$) (Table 2) was 0·38 indicating that only 38% of the variance of the rate values was accounted for by the variable time. For testosterone and benzoic acid, the $r^2$

values (100% and 83%, respectively) showed that the regression line was a useful approximation. The low absorption and subsequently low urine recovery of hydrocortisone gave results below the sensitivity of the analytical methods. The standard deviation obtained is in the same range as reported from earlier experiments in man using the same technique (Feldmann & Maibach, 1969, 1970). A variation of 1–4 times is usual in skin absorption studies and inside the normal biological variation (Wahlberg, 1973).

The results from these guinea-pig studies were encouraging because hydrocortisone and benzoic acid experiments gave values in the same range as the human values; but the different result with testosterone emphasized that an animal model cannot be judged from investigations with few compounds. A broad spectrum of compounds ought to be examined.

The experimental technique using guinea-pigs could perhaps be improved by selecting animals for these studies, because some animals adjust better to the experimental conditions than others. Some animals do not eat enough to keep a constant urine output. In these studies, the urinary output varied from about 20–200 ml/24 h; this increases the experimental error. Modifying cages and collectors might increase the accuracy of the sampling. Increasing the number of animals will also increase precision.

The experiments were performed on the back for practical reasons: (1) the area is protected from the mouth and the legs of the animal and from cage walls; (2) this area allows fixation of the glass ring to inhibit urine contamination by the chemicals falling off or being removed from the skin surface. We suspect that the guinea-pig will show a considerable regional variation as does man (Feldmann & Maibach, 1967) and this problem requires study in guinea-pigs. The amount of radioactive compound which can be wiped and washed off the skin after 24 h of application varies considerably from experiment to experiment, but not as much between the animals in each experiment (Table 3).

The pretreatment of the application site is important to the degree of absorption and must be standardized to compare results from different experiments. Percutaneous absorption of testosterone on the shaved and unshaved forearm of the rhesus monkey gave no significant difference (Wester & Maibach, 1975), while in this study and in an earlier report (Wahlberg, 1972) chemical depilation changed the barrier to increase absorption significantly (Table 3). Six minutes of chemical depilation did not produce visible dermatitis.

The concentration of the compound and the vehicle used constitute other determining factors for the absorption (Wester & Maibach, 1976; Yankell, 1969).

The difference between the wet ashing technique and direct counting of the samples was remarkably small in these experiments and was overwhelmed by the interindividual variation (Table 3). The easier and less time consuming direct counting method gave results in the same range as the wet ashing method. In case of low counts, the wet ashing technique was more accurate and reproducible because of increased counting efficiency and the effective transfer of $^{14}C$ labelled compounds from the sample to the base; burning of greater volumes was also possible. On the other hand, in these guinea-pig experiments, where the main interest was on the total absorption, the direct counting technique proved to be sufficient.

The guinea-pig appears a reasonable rodent model for human skin absorption: it is inexpensive and easy to handle. A suitable urinary output can be maintained on a proper diet. Faeces and urine can be collected and be processed in different ways. More studies must be done to extend this initial study and improve the model.

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# The antiandrogenic effect of progesterone on the hamster flank organ

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## SUMMARY

Progesterone, topically applied, prevents the stimulation of the hamster flank organ by testosterone, but not by dihydrotestosterone. 5α-Dihydroprogesterone does not prevent stimulation by either of the two androgenic hormones. Neither progesterone nor 5α-dihydroprogesterone stimulate the flank organ to any extent.

Implications on the use of progesterone in acne therapy are discussed.

It is currently accepted that 5α-dihydrotestosterone (DHT), which is formed mostly in the target cells *per se* by the 5α-reduction of testosterone, is the active androgen which controls gene expression in the target organ. This mechanism of action has been described for the prostate gland and seminal vesicles (Bruchovsky & Wilson 1968a, b; Stern & Eisenfeld, 1969). It has also been documented that a similar mechanism may be operating in the hamster flank organ (Takayasu & Adachi, 1972), a sebaceous structure, which like the sebaceous gland in the human and other species is androgen dependent. It seems reasonable to assume that inhibition of 5α DHT formation may be an effective means to block androgen action. Therefore Voigt, Fernandez & Hsia (1970) tested a great number of steroids for their capacity to inhibit the conversion of testosterone to 5α DHT in microsomal suspensions of the skin.

Of the two major inhibitors found, progesterone and 4-androstene-3-one 17β carboxylic acid, they examined the effect of the latter compound in the hamster flank organ test. The effect of progesterone on the hamster flank has not been documented yet but it is important to do so since not only has topical progesterone been used in acne therapy, but it has been claimed that 5α-dihydroprogesterone (DHP), which is formed from progesterone in the skin, interacts with the DHT receptor (Wright, Giacomini & Kirchhoffer, 1979). The latter finding may be irrelevant but alternatively it could mean that DHP has some intrinsic androgenic or antiandrogenic activity.

In this paper we describe experiments demonstrating that neither DHP nor progesterone have any androgenic activity. However, progesterone completely prevents the androgenic action of testosterone on the hamster flank organ.

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## MATERIALS AND METHODS

### *Steroids*

The steroids were obtained from Steraloids Inc.

### *The flank organ test*

The flank organ test was essentially as described by Voigt & Hsia (1973). Random bred Syrian golden hamsters, 10 weeks old, were obtained from the Central Institute for the breeding of laboratory animals (Zeist) and fed R, M, H-B from Hope Farms Woerden. At the beginning of treatment and every 3 days thereafter, the animals were closely shaven with an electric hair clipper to expose the flank organ and adjacent skin for visual observation. The female animals were separated into eight groups of five animals each and treated with the various steroids as follows: Group II: testosterone propionate (TP) (4 μg); Group III: TP (4 μg) plus progesterone (P) (400 μg); Group IV: TP (4 μg) plus DHP (400 μg); Group V: DHT (4 μg); Group VI: DHT (4 μg) plus P (400 μg); Group VII: DHT (4 μg) plus DHP (400 μg); Group VIII: DHP (400 μg); Group IX: P (400 μg).

The steroid solution, in 50 μl of acetone/chloroform (75:25) was applied topically on one of the flank organs of each animal while 50 μl of the acetone/chloroform mixture was applied to the contralateral organ which was used as the control. A small pipette was used to deliver the solutions and care was taken that the applied material did not run from the area of the flank organ. Four applications were made every week. The longest diameter of the organs was measured once a week. Statistical significance of the results ($P < 0.05$) was assessed using Student's '*t*'-test.

## RESULTS

The difference in diameter of the flank organs of five male and five female animals is shown in Fig. 1.

In the groups of females treated with testosterone-propionate or dihydrotestosterone on the left

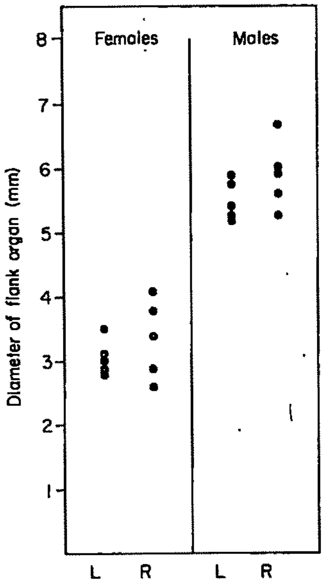

FIGURE 1. Difference in the longest diameter of the flank organs of five untreated female and five untreated male animals.

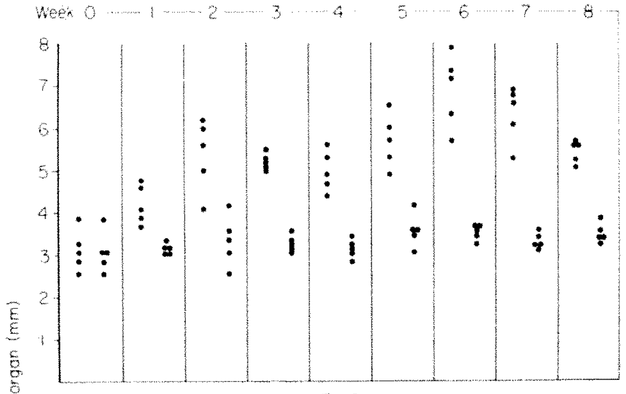

Fig 2

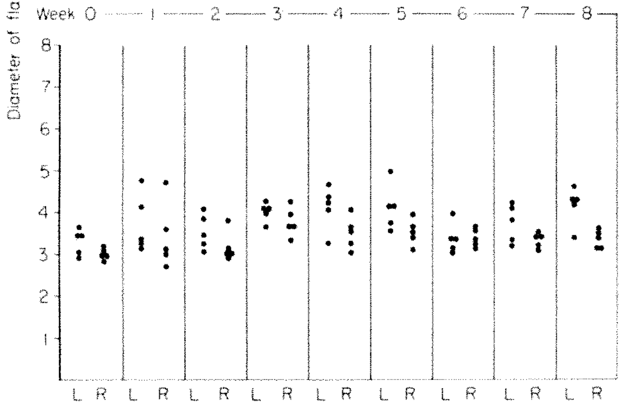

Fig 3

FIGURE 2. Diameters of the left (L, treated) and right (R, control) flank organs of female hamsters as a function of time. Group II: testosterone propionate (4 μg).

FIGURE 3. Group III: testosterone propionate (4 μg) plus progesterone (400 μg). Symbols as Fig. 2.

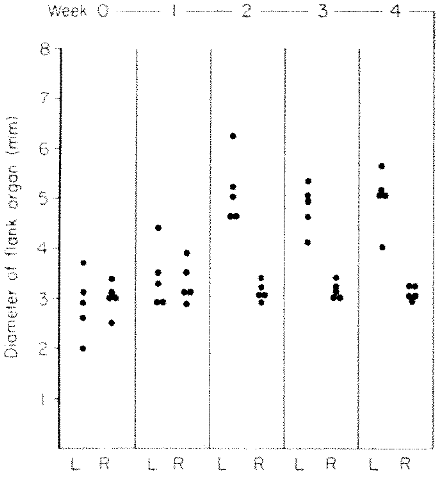

Fig.4

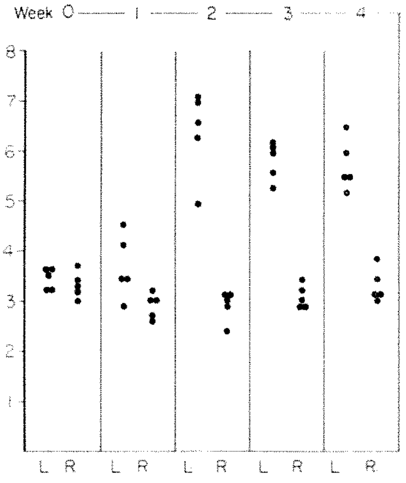

Fig.5

FIGURE 4. Group IV: testosterone propionate (4 μg) plus dihydroprogesterone (400 μg). Symbols as Fig. 2.

FIGURE 5. Group V: dihydrotestosterone (4 μg). Symbols as Fig. 2.

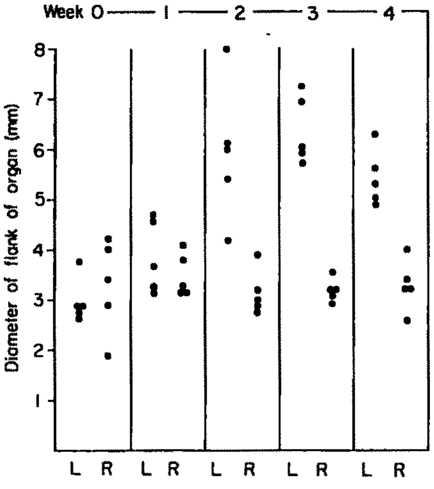

Fig.6

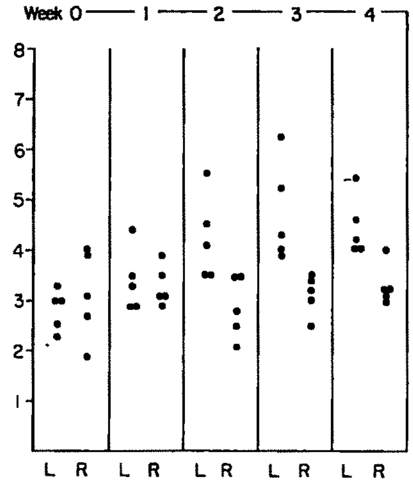

Fig.7

FIGURE 6. Group VI: dihydrotestosterone (4 μg) plus progesterone (400 μg). Symbols as Fig. 2.

FIGURE 7. Group VII: dihydrotestosterone (4 μg) plus dihydroprogesterone (400 μg). Symbols as Fig. 2.

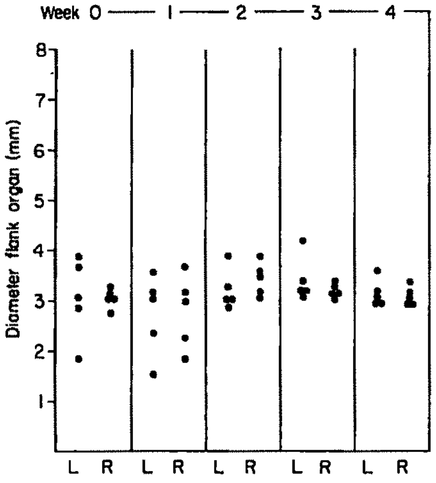

Fig. 8

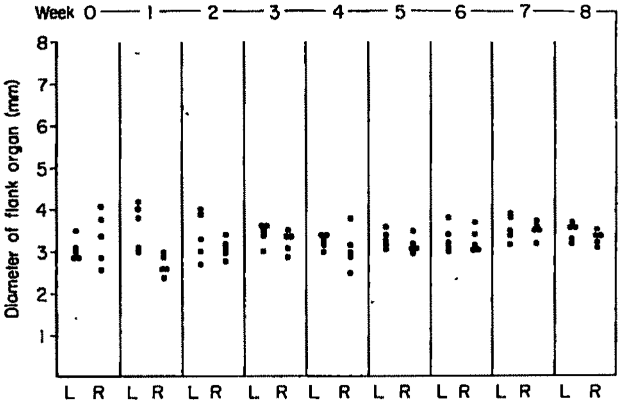

Fig. 9

FIGURE 8. Group VIII: dihydroprogesterone (400 μg). Symbols as Fig. 2.

FIGURE 9. Group IX: progesterone (400 μg). Symbols as Fig. 2.

organ a gradual increase in diameter of the left organs was seen (Figs 2 and 5). The organs on the right side of the animals did not change in diameter, indicating that at the dose used the steroids exert only a local effect. By the end of 3–4 weeks of treatment the treated organs had the same diameter as those of adult males. Progesterone completely blocks the stimulatory action of testosterone propionate ($P<0{\cdot}05$) but not of dihydrotestosterone (Figs 3 and 6), indicating that progesterone exerts its activity by inhibiting testosterone 5α-reductase. Dihydroprogesterone has no influence whatsoever on the stimulatory activity of testosterone propionate or dihydrotestosterone (Figs 4 and 7). Neither dihydroprogesterone nor progesterone exert any stimulatory activity on the flank organs (Figs 8 and 9) ($P > 0·05$). The results for the first 4 weeks are summarized in Table 1.

TABLE 1. Statistical significance (*t*-test) of the variations in size of left (L) flank-organ (treated) compared to right (R) flank-organ (untreated).

| Week | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Groups | L | R | L | R | L | R | L | R | L | R |
| II | 3·06* | 3·03 | 4·12 | 3·10 | 5·28 | 3·28 | 5·14 | 3·20 | 4·88 | 3·10 |
| (TP) | NS | | $P < 0·01$ | | $P < 0·001$ | | $P < 0·001$ | | $P < 0·001$ | |
| III | 3·25 | 3·00 | 3·67 | 3·42 | 3·48 | 3·17 | 3·95 | 3·73 | 4·05 | 3·46 |
| (TP+P) | NS | | NS | | NS | | NS | | NS | |
| IV | 2·86 | 3·01 | 3·41 | 3·31 | 5·13 | 3·13 | 4·78 | 3·16 | 4·95 | 3·09 |
| (TP+DHP) | NS | | NS | | $P < 0·001$ | | $P < 0·001$ | | $P < 0·001$ | |
| V | 3·44 | 3·30 | 3·67 | 2·91 | 6·30 | 2·91 | 5·74 | 3·07 | 5·65 | 3·29 |
| (DHT) | NS | | $P < 0·05$ | | $P < 0·001$ | | $P < 0·001$ | | $P < 0·001$ | |
| VI | 3·02 | 3·28 | 3·90 | 3·51 | 5·94 | 3·15 | 6·34 | 3·19 | 5·41 | 3·29 |
| (DHT+P) | NS | | NS | | $P < 0·001$ | | $P < 0·001$ | | $P < 0·001$ | |
| VII | 2·82 | 3·12 | 3·39 | 3·31 | 4·23 | 2·86 | 4·72 | 3·12 | 4·46 | 3·31 |
| (DHT+DHP) | NS | | NS | | $P < 0·02$ | | $P < 0·01$ | | $P < 0·01$ | |
| VIII | 3·10 | 3·11 | 2·78 | 2·82 | 3·25 | 3·46 | 3·43 | 3·23 | 3·17 | 3·13 |
| (DHP) | NS | | NS | | NS | | NS | | NS | |
| IX | 3·07 | 3·36 | 3·62 | 2·71 | 3·38 | 3·10 | 3·23 | 3·25 | 3·25 | 3·08 |
| (P) | NS | | $P < 0·01$ | | NS | | NS | | NS | |

* Mean value in mm of L or R flank-organ of the five hamsters of each group. NS = not significant ($P \geq 0·05$).

## DISCUSSION

Androgens play a part in the production of acne by stimulating formation of sebum, and by causing hyperkeratinization of the sebaceous follicle. It is now generally believed that 5α-dihydrotestosterone is responsible for stimulation of the sebaceous gland (Takayasu & Adachi, 1972) and in acne the evidence for increased local formation of dihydrotesterone has been documented (Sansone & Reisner, 1971).

Our results clearly show that topically applied progesterone can inhibit the 5α-reduction of testosterone. Moreover, it is demonstrated that progesterone does not exert any androgenic effect. At a concentration of 100 times that of TP or DHT no significant androgenic or antiandrogenic activity could be found for dihydroprogesterone. We may therefore conclude that the local formation of DHP in the skin is most probably not involved in the decrease of the effect of progesterone after 3 months of continuous application (Simpson *et al.*, 1979) in humans, which makes intermittent application necessary.

It has recently been claimed that dehydroepiandrosterone may be more sebotrophic than dihydrotestosterone (Cooper *et al.*, 1979). We have shown that progesterone, an inhibitor of sebum secretion in at least women (Simpson *et al.*, 1980), does not influence dehydroepiandrosterone metabolism (Vermorken, Goos & Roelofs, 1980). Therefore, at least in women dihydrotestosterone must be regarded as an important physiological sebotrophic factor.

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***Case Reports***

# Acute and chronic graft-vs-host reaction in skin: report of two cases*

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## SUMMARY

The cutaneous changes of the acute form of graft-vs-host reaction are described in two patients who underwent bone marrow transplantation for treatment of severe aplastic anaemia. One patient went on to develop a chronic reaction of the lichenoid type. Histopathology confirmed the presence of 'satellite cell necrosis' from the acute stage of the disease onwards. Direct immunofluorescence showed deposits of immunoglobulin (IgG, IgM), fibrinogen and complement on the necrotic keratinocytes, whilst the basement membrane was positive for C3. These findings suggest that humoral immunity as well as cellular immunity may play a part in the production of the rash.

Graft-vs-host reaction (GVHR) occurs whenever marrow or other tissue containing immunologically competent allogeneic cells is infused into a host unable to reject the donor cells.

Recently, bone marrow transplantation has been used with increasing frequency for treatment of haematological disorders such as aplastic anaemia, acute leukaemia, genetic disorders and solid tumours (Slavin & Santos, 1973). This procedure provides the optimal clinical setting for the development of GVHR in humans; in fact, GVHR has become one of the major complications of this procedure, occurring in approximately 70% of recipients of allogeneic bone marrow transplantations (Masters, Hood & Cosoni, 1975). The pathophysiology of the disorder remains unclear, especially the contribution of the host to the disease (Slavin & Santos, 1973). The effector cell mediating GVHR is probably a T cell, but involvement of other cell populations is likely. For unknown reasons, GVHR tends to manifest in target organs in man, especially skin, liver and gut (De Dobbeleer, Ledoux-Corbusier & Achten, 1975; Masters, Hood & Cosoni, 1975; Hood *et al.*, 1977). The dermatological manifestations can be divided into acute and chronic phases (Hood *et al.*, 1977). Acute GVHR tends to occur between 7 and 21 days after transplantation and is characterized by a macular, erythematous eruption, usually beginning on the trunk and spreading to the face and extremities. A more acute and severe reaction has been reported also (Masters *et al.*, 1975). This reaction may occur within the first 6

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weeks after transplantation and is characterized by localized or diffuse exfoliative dermatitis, bullae or even toxic epidermal necrolysis (Peck, Herzig & Elias, 1972). Chronic GVHR occurs several months to a year after transplantation; less is known about this phase because of the small number of long term survivors. Lichenoid eruptions (Peck *et al.*, 1972; Saurat *et al.*, 1975a, b; Thomas *et al.*, 1975), poikiloderma (Slavin & Santos, 1973; Thomas *et al.*, 1975) and scleroderma like changes (Slavin & Santos, 1973; Touraine *et al.*, 1975; Hood *et al.*, 1977; Lawley *et al.*, 1977) have been described.

The purpose of this paper is to report two cases of acute GVHR following bone marrow transplantation, the second case evolving to a lichenoid pattern typical of chronic GVHR.

## CASE REPORTS

### *Case 1*

A 36-year-old man underwent an allogeneic bone marrow transplant for treatment of severe aplastic anaemia. The donor was an HLA-identical (mixed lymphocyte culture (MLC)-negative) sister. Engraftment was confirmed by restoration of haematological parameters and by the presence of a graft marker, in this case a sex mismatch, the karyotype 44XX being found in the bone marrow of the male recipient 30 days post-operatively.

Three days later the patient developed an erythematous macular rash on the trunk that spread to the face and extremities. This was associated with vomiting, diarrhoea and evidence of liver involvement. A biopsy of this rash was taken the following day and processed for conventional histology only. The patient later died from interstitial pneumonia due to cytomegalovirus.

### *Case 2*

A 22-year-old man also underwent an allogeneic bone marrow transplant for severe aplastic anaemia. The graft was obtained from an HLA-identical (MLC negative) brother and transplantation was followed by rapid engraftment and restoration of haematological parameters. Engraftment was confirmed by some minor differences in blood groups on the eightieth post-operative day.

Thirty-six days after transplantation the patient developed an erythematous macular rash on the trunk that spread to the face, neck and extremities. There was no clear-cut evidence of liver or gastrointestinal involvement.

Following this, on the eightieth post-operative day, with the patient still receiving weekly methotrexate as prophylaxis against a GVHR, a severe lichen planus-like eruption appeared over the palms, dorsa of the hands, arms and trunk. Severe and disabling erosions developed on the oral mucosa, especially the palate and lips. A scaling dermatitis appeared over the face and neck.

Sixty days later the lichen planus-like lesions on the skin had become slightly atrophic and a brownish reticulate pigmentation had appeared. The oral lesions had improved, and at that time there was scaling on the lips only. During the three different stages of skin involvement, biopsies were obtained and examined by both light and immunofluorescence microscopy.

### *Histology*

Biopsies for conventional histopathology were fixed in Bouin's solution, paraffin embedded and stained with haematoxylin-eosin, Masson's trichrome and PAS.

Biopsies for direct immunofluorescence were incubated with Boehringer rabbit antihuman sera conjugated with fluorescein (IgA, IgG, IgM, C3 and fibrinogen) and observed with a Reichert Fluorpan epifluorescence microscope (absorption filters FITC and OG515). They were photographed with a Kodak Ektachrome 160 Asa-tungsten film in a Leitz Dialux epifluorescence microscope with an Orthomat incorporated camera.

Indirect immunofluorescence studies were also carried out, incubating sections of rabbit lip with the patient's serum and afterwards using the same antihuman rabbit sera.

*Light microscopy.* The histopathological changes in the first patient and in the first phase of the second patient were identical.

Whilst the papillary dermis had only a sparse, diffuse infiltrate of mononuclear cells, an extensive lymphocytic exocytosis was present. In the areas of lymphocytic invasion, spongiosis and discrete acantholysis were prominent, but a characteristic feature was the presence of many degenerate keratinocytes, each with a pyknotic nucleus and eosinophilic, hyalinized cytoplasm (Fig. 1). These necrotic cells were associated with one or more satellite lymphocytes ('satellite cell necrosis') (Fig. 2). Where areas of hyperkeratosis were observed, eosinophilic, hyalinized bodies were occasionally seen high up in the epidermis.

In the lichenoid lesions of the second patient, a mild, band-like, lymphocytic infiltrate was present in the upper dermis with severe vacuolization of the basal layer and small areas of dermo–epidermal splitting. Sparse, single, necrotic epidermal cells associated with satellite lymphocytes were seen only near the basal layer. Hyperkeratosis and hypergranulosis were prominent findings. The PAS stain showed a wrinkled basal membrane.

The pigmented lesions in the second patient showed an atrophic epidermis without satellite cell necrosis. In the papillary dermis there was a sparse mononuclear infiltrate and large amounts of melanin in macrophages. There was no dermal fibrosis. The picture was similar to that seen in residual lesions of lichen planus after the papules have flattened.

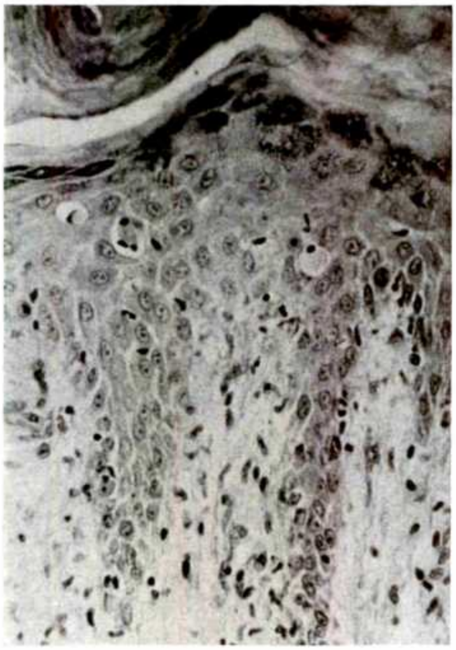

FIGURE 1. Case 2. Lymphocytic exocytosis. 'Satellite cell necrosis' in the Malpighian layer.

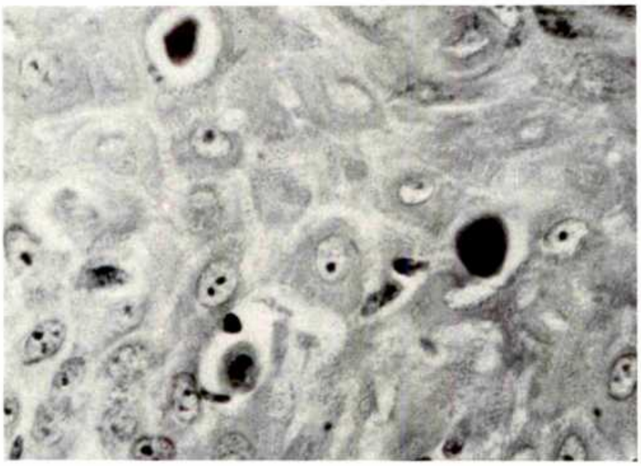

FIGURE 2. Case 2. 'Satellite cell necrosis'. Necrotic keratinocytes associated with lymphocytes.

*Electron microscopy*. The findings confirm, in general, the observations by Woodruff, Butcher & Hellestein (1972) in animals, and by Grogan, Odom & Burgers (1977) in humans; these EM studies are reported in detail in another paper (Rozman *et al*., 1979).

*Immunofluorescence*. Direct immunofluorescence, carried out only in the second case, was done on the erythematous and lichenoid lesions and the results were similar. Whilst the necrotic keratinocytes stained homogeneously for IgG, IgM, fibrinogen and C3, the basement membrane was strongly positive for fibrinogen only, and mildly positive for C3 (Fig. 3). Indirect immunofluorescence studies were negative.

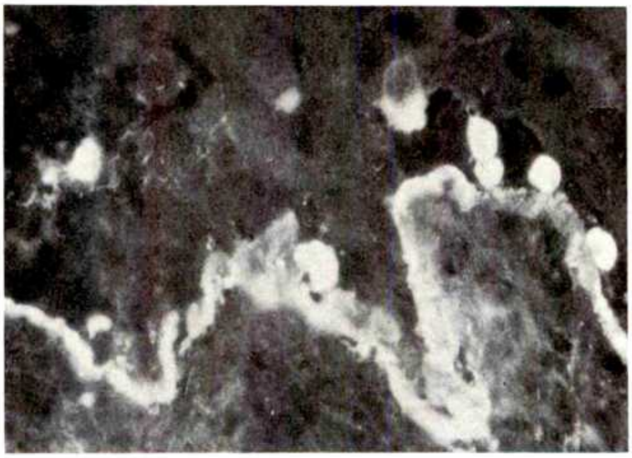

FIGURE 3. Case 2. Direct immunofluorescence. Fluorescence of the necrotic keratinocytes and the basement membrane (antihuman fibrinogen).

## DISCUSSION

Awareness of the cutaneous manifestations of GVHR is very important for early diagnosis of this condition, as well as for the surveillance of its course.

Our histological findings show that 'satellite cell necrosis' can be found from the early stages of skin GVHR. Although not absolutely specific, this lesion seems to be very characteristic of GVHR (Woodruff *et al.*, 1972; Grogan *et al.*, 1977; Claudy, Schmitt & Freycon, 1979). Isolated necrotic keratinocytes are not a specific phenomenon and they have been seen in a great number of cutaneous disorders, especially in patients treated by hydroxyurea and/or other cytostatic drugs (Smith & Gelfant, 1974; Kennedy, Smith & Goltz, 1975). The lack of lymphocytes related to altered epidermal cells differentiates these appearances from 'satellite cell necrosis'. Recently Gange & Levene (1979) have emphasized the striking similarity between 'satellite cell necrosis' of GVHR and the necrotic keratinocytes seen in oxprenolol eruptions, although in their case identification of lymphocytes specially related to necrotic epidermal cells was difficult (Gange & Levene, 1979).

During the acute phase of GVHR 'satellite cell necrosis' can be detected at any level of the epidermis, including the granular layer and the stratum corneum, resulting in transepidermal elimination of the hyalinized cellular elements.

If the patient succeeds in overcoming the acute period of GVHR, as we were able to observe in one of our cases, the cutaneous manifestations may change from a non-specific erythematous rash to a more definite lichenoid eruption (Saurat *et al.*, 1975a, b; Touraine *et al.*, 1975). In addition, a sclerodermiform pattern has also been referred to. During this chronic GVHR, lesions of the oral mucosa may be seen, either reticulate or only erosive (Saurat *et al.*, 1975a, b). The latter was the case in our second patient.

The lichenoid lesions tend to fade and to become progressively atrophic and pigmented, as may happen in lichen planus. This appearance should not be confused with genuine poikiloderma, which may be observed *de novo* in cutaneous GVHR. In the latter condition, atrophy and pigmentation are associated with telangiectasia, which is lacking in the former.

The lichenoid phase of chronic GVHR is characterized by progressive disappearance of 'satellite cell necrosis' from the upper epidermal layers, tending to be sparser than in the acute period and localized to the basal layer. This localization and the similarity between 'satellite cell necrosis' and Civatte bodies may be reasons why this type of lesion has apparently been overlooked by some authors.

Our immunological findings deserve special consideration. In a case of chronic GVHR with a sclerodermiform pattern, Spielvogel, Goltz & Kersey (1977) found deposits of IgM at the dermo-epidermal junction, as well as granular deposits of IgM, IgA and C3 in the dermal vessel walls. By an *in vitro* technique, Merrit, Mann & Rogentine (1971) showed in the serum of patients with GVHR, the presence of IgM cytotoxic antibodies against epithelial cells. Our observation of necrotic keratinocytes staining with immunoglobulin has not been reported previously. The possibility that the phenomenon might represent non-specific absorption of immunoglobulins by the necrotic cells is not very likely, since the deposits were limited to IgG and IgM, whereas IgA was repeatedly lacking. With non-specific absorption, the presence of all classes of immunoglobulins would be expected. The involvement of cellular immunity in the pathogenesis of GVHR seems well established. But our findings would support, as certain authors believe (Spielvogel *et al.*, 1977), that humoral immunity might also play a role.

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# A distinctive skin eruption following small-bowel by-pass surgery

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## SUMMARY

Crops of distinctive skin lesions appeared after jejuno-ileal by-pass surgery for morbid obesity in three patients. They consisted mainly of large numbers of macules on the extremities, of vague outline and with a tendency to central pustulation and necrosis. Severe arthralgia and myalgia preceded the skin lesions, which histologically showed a dense neutrophil leukocytic infiltrate, nuclear debris, and fibrin deposition around blood vessels in the dermis.

Jejuno-ileal by-pass has become a popular operation for the treatment of morbid obesity. Unfortunately, complications such as arthritis, diarrhoea, flatulence, electrolyte imbalance, liver failure, urinary tract stones, psychiatric disturbances and malabsorption may follow the procedure (Halverson *et al.*, 1978). Several vitamin deficiencies have been observed including a young woman with vitamin A deficiency who presented with phrynoderma (Wechsler, 1979). Protein malnutrition may cause hair loss, a dry scaly skin and oedema. Serum from patients with acute arthritis contained cryoprotein complexes which were thought to be important in the pathogenesis of the arthritis (Wands *et al.*, 1976). Liver and kidney biopsies, from a patient with circulating cryoglobulins, have also revealed granuloma formation (Sweet *et al.*, 1978). Goldman *et al.* (1979) described two jejuno-ileal by-pass patients who had crops of erythematous macular, papular and vesiculo-pustular lesions associated with inflammatory joint disease. The skin lesions showed leukocytoclastic vasculitis.

Drenick *et al.* (1976) reported that skin lesions were noted in four of their twenty-six patients. Small, tender erythematous nodules or typical erythema nodosum lesions occurred singly or in crops over arms, legs and abdomen. In one of their patients the lesions cleared after re-anastomosis of the by-pass.

Hardy (1978) found skin complications in eight out of approximately 200 patients who had undergone by-pass surgery. He described three types of skin lesions: clear vesicles on normal skin, pustules on normal skin, and vesicles and pustules with a flare of erythema. The histopathological patterns seen were a lymphocytic perivascular infiltrate, subepidermal vesicles, and dermal and epidermal necrosis with a lymphocytic perivascular infiltrate. After immunofluorescent examination of the lesions, which

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disclosed positive basement membrane fluorescence with IgG and complement, he made a tentative diagnosis of bullous pemphigoid.

Of approximately 180 patients who have undergone by-pass surgery in Pretoria, three developed subsequent recurrent skin lesions. Skin biopsies and serological examinations were carried out during the active and quiescent phases.

## RESULTS

### *Patients*

Case 1 was a woman of 58 who had undergone a jejuno-ileal by-pass operation for obesity 2½ years earlier. Thereafter for 6 months she suffered from rigors, arthritis and myalgia. The first skin lesions appeared 1 year after the by-pass procedure. Arthralgia and myalgia always preceded the skin lesions by 1 and 2 days. The attacks recurred more or less at monthly intervals. The skin lesions appeared mainly on the extensor surfaces of the limbs, and started as erythematous macules which developed central pustulation or necrosis after a day or two. The lesions, numbering between twenty and 100, reached 25 mm in diameter with a flare of erythema that faded and disappeared peripherally (Fig. 1). Individual lesions lasted 4 to 5 days, and attacks up to 8 days. There was no scarring.

Cases 2 and 3 were women, aged 39 and 26. Their skin lesions appeared for the first time 2 years after by-pass surgery. These were similar to those of Case 1, being also preceded by myalgia and arthralgia and appearing mainly on the extremities and neck. Both these patients developed erythema nodosum on the lower legs simultaneously with the 'by-pass skin lesions' (Fig. 2), although it did not accompany every crop of these lesions.

### *Histopathology of the skin lesions*

Many biopsies were obtained from all three patients during different attacks. The main feature was a massive neutrophil leukocytic infiltrate in the upper dermis. In some sections there was also a leukocytoclastic vasculitis, with fibrin deposition around blood vessels and nuclear debris (Fig. 3). In early lesions, which clinically consisted only of erythematous macules, the epidermis appeared normal. More advanced lesions, with central pustulation, showed epidermal degeneration above the inflammatory process in the dermis. Subepidermal oedema and fibrin deposition were also seen, but always in association with a massive underlying neutrophil leukocytic infiltrate (Fig. 4).

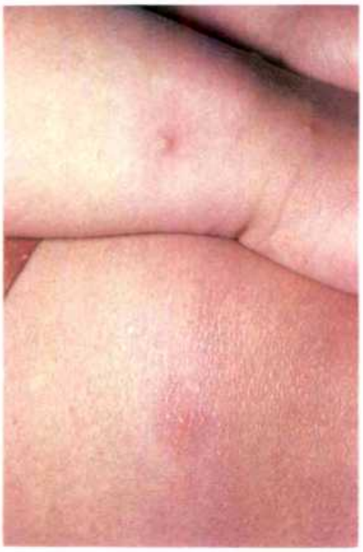
On immunofluorescent examination all three patients showed fibrin deposition in the entire area of inflammation, and especially in the subepidermal oedematous zone. No specific deposits of immunoglobulins IgG, IgA, IgM or of complement were observed.

Biopsies taken from the erythema nodosum-like lesions showed minimal dermal changes but revealed an infiltrate in the fat layer consisting of accumulations of mainly lymphoid cells.

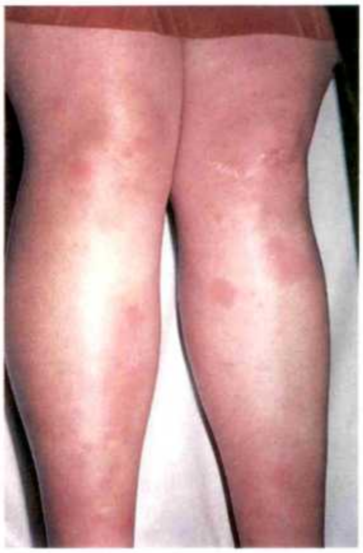
### *Special investigations*

*Trafuril patch test reactions.* Topically applied tetrahydrofurfuryl ester of nicotinic acid 5% in an ointment (Trafuril, Ciba, Basel) produced skin lesions 24 h after application during two attacks in one untreated case. No abnormal reactions were observed in the other two patients who were receiving dapsone and prednisone. The histology of the Trafuril-produced lesion was that of a massive polymorph neutrophil infiltrate in the dermis, with nuclear debris and a possible vasculitis.

*Cryoglobulins.* Cryoglobulins of a mixed IgG/IgM type were demonstrated in the serum of Case 1 in an acute exacerbation.



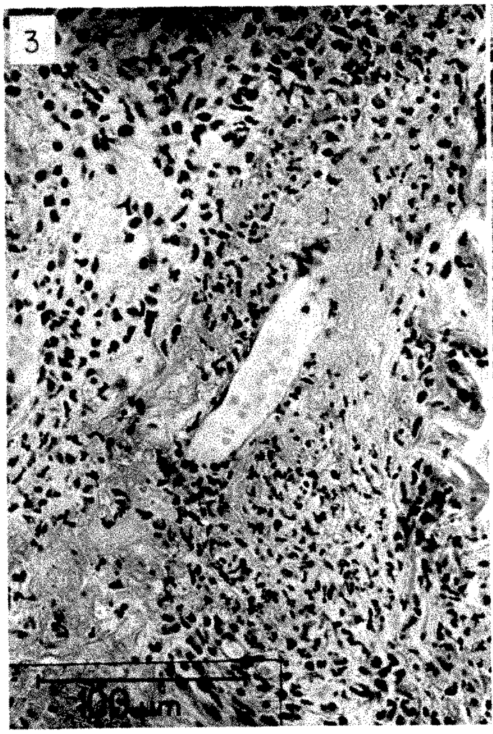
(1)



(2)

FIGURE 1. Erythematous lesions with an area of central pustulation on the forearm and thigh. Case 3.

FIGURE 2. Erythema nodosum-like as well as erythematous lesions with central pustulation, in the knee area. Case 3.



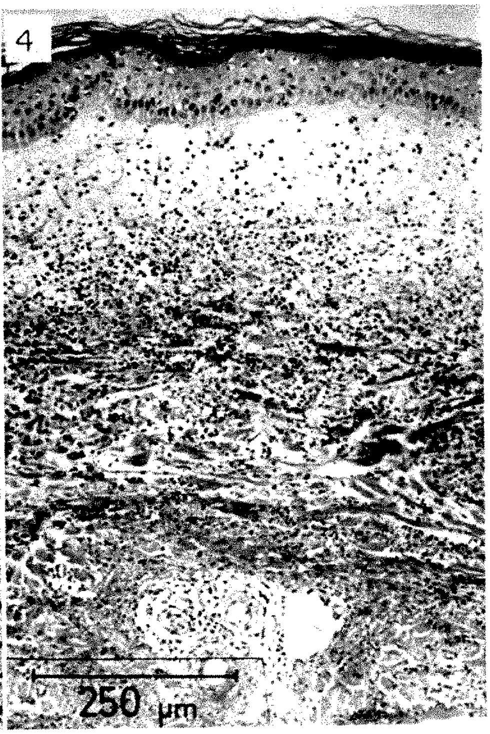


FIGURE 3. Neutrophil leukocytes, nuclear debris and fibrin around blood vessels. Case 1.

FIGURE 4. Subepidermal oedema and fibrin deposition with masses of underlying neutrophils and nuclear debris. Case 1.

*Bacterial studies.* Repeated blood cultures taken during episodes of myalgia and arthralgia, and cultured aerobically and anaerobically, failed to yield any organisms. The same negative results were obtained from swabs taken from skin lesions.

Strains of *Bacteroides fragilis* and *Escherichia coli* were isolated from the stools of a patient and used as antigens to measure the antibody titre of the serum antibodies by indirect immunofluorescence. Although antibodies could be demonstrated in titres varying from 1/20 to 1/320 similar titres were found in the sera of five healthy controls matched for age and sex.

*Complement studies.* The complement C1q values were normal. The total complement C3 concentration was increased, but about 10% of this total, as shown by two-dimensional immuno-electrophoresis, consisted of C3 degradation products. Serum anticomplementary activity was consistently present. No auto-antibodies directed to tissue antigens could be demonstrated, using mouse stomach, kidney, and liver as substrates for indirect immunofluorescence.

*Electrophoresis.* Serum electrophoresis on cellulose acetate showed a decrease in albumin and an increase in the gamma-globulins. The latter, on immuno-electrophoresis, represented a polyclonal increase in all immunoglobulins.

*Other findings.* The ESR and C-reactive protein values were consistently raised. The geometric mean values found for the ESR and C-reactive protein were: Patient 1, ESR 50 mm, CRP 30 mg/l; Patient 2, ESR 25 mm, CRP 40 mg/l; Patient 3, ESR 45 mm, CRP 100 mg/l.

*Therapy*

Dapsone, 50 mg daily, alleviated symptoms and decreased the frequency of attacks. The addition of prednisone (5–10 mg on alternate mornings) helped control skin lesions and prevent attacks.

Recently we have used metronidazole (400 mg twice daily for 5 days) which aborted attacks when given early, decreasing the necessity for dapsone and prednisone therapy and confirming the findings of Drenick *et al.* (1976).

Re-anastomosis of the intestinal shunt in Case 3 resulted in the disappearance of cutaneous and associated symptoms.

## DISCUSSION

In three of our patients intestinal by-pass surgery led to skin lesions with a striking clinical and histological uniformity that we feel is distinctive. The lesions cannot be confused with bullous pemphigoid despite the basement membrane fluorescence described by Hardy (1978), a finding we have failed to confirm. Histologically it recalls Sweet's febrile neutrophilic dermatosis but it cannot readily be confused clinically with Sweet's syndrome.

The pathogenesis of the cutaneous lesions remains speculative. The 'blind' ileal segment has been shown to harbour faecal bacteria, and chronic inflammatory changes have been demonstrated in the gut wall histologically (Drenick *et al.*, 1976). The constantly and considerably raised ESR and C-reactive protein values suggest an inflammatory reaction which is continuously present. Success in preventing the attacks with metronidazole points to an infective agent playing a role. We failed, however, to culture bacteria from either skin lesions or blood samples. These findings suggest that the lesions are not caused by dissemination of living bacteria.

We may speculate that whatever the intestinal antigen may be, its absorption is aided by the chronic mural inflammation. The small intestine comes into contact with a diversity of antigens from food during digestion, as well as an inexhaustible variety of metabolites from the resident bacterial flora. Normally the body handles all of these without mishap. Following by-pass surgery the 'blind' ileal loop constitutes a sequestered compartment which favours chronic mural inflammation and absorption of antigens from the lumen. This absorption is only periodic, which may account for the intermittent attacks. Once absorbed, circulating immune complexes are formed which, as in Case 1, may be cryoprotein complexes. These immune complexes may then be deposited in blood vessels giving rise to the leucocytoclastic vasculitis observed in skin lesions which are associated with attacks of arthritis.

## ACKNOWLEDGMENTS

We wish to thank the S.A. Medical Research Council for their support.

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*British Journal of Dermatology* (1980) **102,** 473.

***Society Proceedings***

# Proceedings of the 215th Meeting of the Netherlands' Society of Dermatologists, Groningen, 12 May 1979

PRESIDENT: DR M.J.WOERDEMAN

HOST: PROFESSOR A.H.KLOKKE

## SUMMARIES OF PAPERS

### Immunohistochemical findings in a patient with penicillamine pemphigus

M.C.J.M.DE JONG

*Department of Dermatology, Groningen, The Netherlands*

This paper has been published in full in *British Journal of Dermatology*, **102,** 333.

### Protease inhibitor deficiencies in a patient with angio-oedema: results of family studies

E.BLEUMINK and H.M.G.DOEGLAS

*Department of Dermatology, Groningen, The Netherlands*

Evidence has been presented that protease inhibitor deficiencies may play a role in the pathogenesis of chronic urticaria and angio-oedema. Protease inhibitors are proteins capable of inhibiting proteolytic enzymes such as plasmin, kallikrein, C1-esterase, leukocytic and tissue proteases, trypsin and chymotrypsin.

If mediator systems (the complement, kallikrein or clotting/fibrinolysis systems) are activated (for example, by immunological reactions or by tissue injury) they inhibit the activated enzymes. Where there is an inhibitor deficiency an overflow of one or more mediators (kinins, anaphylatoxins) may occur. This is the case in patients with hereditary angio-oedema, whose plasma is deficient in C1-esterase inhibitor.

In earlier studies of a group of 150 patients with chronic urticaria markedly decreased levels of protease inhibitors were found in patients with cold contact urticaria (Doeglas & Bleumink, 1975). Six out of nine patients with angio-oedema showed deficiencies of alpha$_1$-antitrypsin, antichymotrypsin or anti-kallikrein activity (Bleumink & Doeglas, 1977). In the present paper the data are described of a 30-year-old female patient with recurrent angio-oedema. The patient showed a deficiency of alpha$_1$-antitrypsin ($<20\%$ of the normal value) as well as of antichymotrypsin ($<10\%$ of the normal value). No abnormalities were found of the complement, kallikrein or fibrinolytic systems. Phenotyping of alpha$_1$-antitrypsin revealed a ZZ type, while the parents both had a MZ type.

Clinical and laboratory investigations of twenty-one family members showed:

(1) A marked decrease of alpha$_1$-antitrypsin in fourteen individuals; six of these had antitrypsin levels $<50\%$ of the normal value;

(2) An antichymotrypsin deficiency in eight out of sixteen cases;

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(3) Chronic urticaria in three individuals, while two were found to have emphysema; liver cirrhosis could not be detected;

(4) The three patients with chronic urticaria had a protease inhibitor deficiency as well.

These investigations combined with earlier results suggest that individuals with a protease inhibitor deficiency have a predisposition to develop chronic urticaria and angio-oedema.

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## Dystrophia bullosa hereditaria, typus maculatus (Mendes da Costa–van der Valk): a rare genodermatosis

J.H.HASSING and H.M.G.DOEGLAS

*Department of Dermatology, Groningen, The Netherlands*

In 1908 Mendes da Costa and van der Valk described a genodermatosis in two brothers from a family living in Amsterdam. The disease was inherited recessively, occurred exclusively in male members of the family and was transmitted by clinically healthy women. At birth the patient showed microcephaly. During the third month of life complete atrichia developed together with a typical reticular red-brown pigmentation on the face and extremities. The involved skin showed recurring blisters without previous trauma. This symptom usually disappeared spontaneously after the third year of life. All the patients died at an early age from infections or squamous cell carcinoma.

Several other cases were described in later generations (Carol & Kooy, 1937; Woerdeman, 1957) but never outside this family.

In the summer of 1977 we saw an 11-month-old male child with microcephaly, complete atrichia, spontaneous blister formation and extensive reticular pigmentation of face and extremities (Fig. 1). The mucous membranes were normal. At the age of 6 months he had been admitted to the hospital for a pulmonary infiltration. His mother was descended from the family in Amsterdam in which the disease had been described originally.

Because the last paper dated from 1957, it was possible to review the disease with newer techniques. We were interested in the histopathology of the blisters, immunofluorescence findings of the skin, possible immunological disturbances responsible for the deaths from infectious disease and possible chromosomal abnormalities.

The patient showed considerable growth retardation: his length and weight remained under the 10 percentile line, and his skull diameter was far below the 10 percentile line. The skull showed a normal thickness and bone structure on X-ray; the sella was normal in configuration. At the age of 2 years the skull sutures had not closed. There were no signs of increased intracranial pressure. It was concluded that the microcephaly was based on underdevelopment of the cerebrum.

His motor development was normal for his age, but his mental functions remained backward. His total development quotient was slightly below normal. Histopathological examination of a blister showed, with haematoxylin–eosin staining, an intraepidermal blister, intracellular oedema and vacuolization of the basal cells in the area surrounding the blister.

A modified Bloch stain showed pigmentation in the upper layers of the cutis. Immunofluorescent staining of lesional skin repeatedly showed IgM-positive cytoid bodies subepidermally. The non-involved skin did not show any abnormalities. Laboratory values did not show significant changes. In particular there were normal values for IgG, IgA, IgM, IgE, the complement factors C4, C3 and the

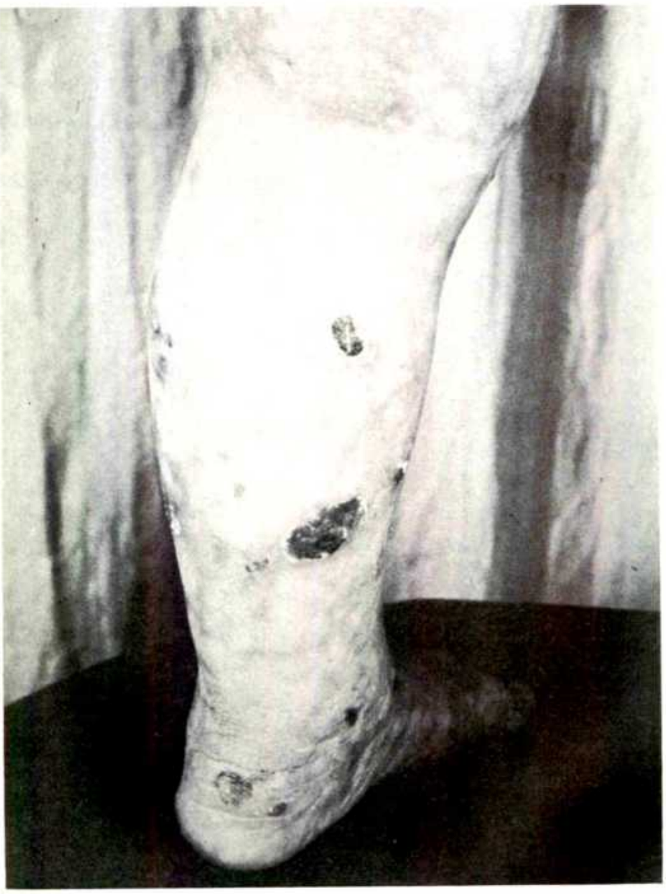

FIGURE 1. Dermatitis bullosa hereditaria, typus maculatus. Reticular pigmentation and bullae legs.

C1 INH. There was a negative ANA test, and there were no circulating antibodies against intercellular substance of epidermis, gastric parietal cells, mitochondria, reticulin or smooth muscle. There were normal values for alpha$_1$-antitrypsin and normal trypsin, chymotrypsin and kallikrein inhibition. Blister fluid was sterile on bacteriological culture. Immunological tests such as vaccination with RS virus gave a normal rise in serum antibodies, DNCB sensitization gave a normal response, the PHA stimulation of lymphocytes was normal. The leukocyte function as tested with a nitro blue tetrazolium test was normal. A bone marrow smear showed no abnormalities. Study of the chromosomes showed a normal male karyotype. In a lymphocyte culture there was the same frequency of sister chromatid exchange as in a control culture.

In summary no new point of view came to light from the histopathological and immunofluorescence examinations of the skin. The search for disturbances in immunological defence mechanisms did not show serious defects. Preliminary studies of the chromosomes did not show overt abnormalities.

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## A dental aspect of tuberous sclerosis

M.HOFF

*Department of Pedodontics, University of Groningen, The Netherlands*

Dentists treating mentally retarded patients must not only care for the patient's dental health, but also be aware of the presence of orofacial abnormalities that can be of diagnostic value.

Epilepsy, mental retardation and 'adenoma sebaceum' are the main diagnostic features of the fully developed form of tuberous sclerosis. Next to this triad, lesions can be found elsewhere in the skin, eyes, viscera and skeleton. The expression of the syndrome, however, is variable and there are incomplete forms that cause difficulty in recognizing the disease.

During dental treatment of six patients with tuberous sclerosis, in an institute for mentally retarded patients, the occurrence of multiple defects in tooth enamel was noticed. All teeth of all the six patients showed pit-shaped enamel defects. There were about three pits per tooth surface, varying in diameter from 0·1 to 100 $\mu$m. All surfaces were affected at random. The enamel defects were detectable radiographically, especially in the anterior teeth, as pin-sized radiolucencies. Scanning electron microscopy was used to study the detailed structure of the enamel surface as well as the defects.

The clinical picture of the enamel hypoplasias associated with tuberous sclerosis gave the impression of a disturbance occurring throughout the whole of amelogenesis. This has been confirmed by scanning electron microscopy.

The enamel defects seem to be pathognomonic of tuberous sclerosis and may be important in the early diagnosis of this complex syndrome.

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## Contact allergic reactions to fragrance chemicals

J.P.NATER (representing the Dutch Contact Dermatitis Research Group)

A group of fourteen dermatologists from different parts of The Netherlands, in cooperation with seven university clinics, conducted an investigation on the occurrence of contact allergic reactions to fragrance components used in perfumes. During a period of 1 year 192 persons suspected of perfume allergy were patch tested with:

(1) The standard series of the International Contact Dermatitis Research Group (twenty allergens).
(2) A series of twenty-two fragrance chemicals.

Eighty-two of the tested persons reacted positive to one or more of the fragrance chemicals. In many cases (38·7%) the contact dermatitis was primarily located on the hands, the clinical picture being indistinguishable from a contact dermatitis of the hands caused by other allergens. The most important allergens were:

coumarine: 7% of the group reacted positive;
hydroxycitronellal: 10·6% positive;
cinnamic alcohol: 10·6% positive;
eugenol: 11·2% positive.

The indicator allergens colophony, wood tar, Balsam of Peru and turpentine gave the following results. Of the patients reacting to colophony, 60% reacted also to one or more fragrance materials. For the other three indicator allergens the percentages were: wood tar: 72%; Balsam of Peru: 79%; turpentine: 70%. From the group of patients reacting negative to these four indicator allergens 33·6% still reacted positive to one or more perfume substances.

Thus patch testing with fragrance chemicals appeared to be a valuable addition to the patch test series used to diagnose cases of perfume allergy.

Further details will be published in *Contact Dermatitis*.

## Report of the Dutch Mycosis Fungoides Study Group

L.HAMMINGA AND W.A.VAN VLOTEN

*Department of Dermatology, University Hospital, Leiden, The Netherlands*

A multi-centered study group on mycosis fungoides has been active since 1975 in The Netherlands. Since that time a uniform staging procedure has been used after the diagnosis of MF has been established and patients are staged according to a modified Fuks staging classification. After staging, every centre chooses one of the protocols for treatment depending on the stage of the disease. The protocols for treatment of Stage I and Stage II are:

(1) topically applied nitrogen mustard (HN2);
(2) total skin electron beam irradiation (E total skin);
(3) oral photochemotherapy (PUVA).

The protocols for MF Stages III and IV are:

(1) COP scheme (cyclophosphamide, oncovin, prednisone);
(2) MOPP scheme (mechlorethamine, oncovin, procarbazine, prednisone).

These schemes are often combined with one of the above mentioned topical regimens.

In 1978, 110 patients with MF were treated, sixty-eight males and forty-two females of which twenty were new patients (in 1977: twenty-four). In that year eight patients died. In one case death was not related to the disease. In this group of patients the mean duration of skin lesions before the diagnosis could be made was 9·1 years. In most patients the diagnosis was made in the sixth and seventh decade of life.

TABLE 1. Number of patients per stage and results of therapy

| Stage | Premycotic A | Plaques B | Tumours C | Total | No. of patients who died in 1978 | Mean follow-up (years) |
|---|---|---|---|---|---|---|
| I. MF of the skin | 8 (7)* | 30 (24) | 10 (4) | 48 (35) | 2 | 3 |
| II. MF of the skin with DLAP | 7 (6) | 26 (19) | 9 (4) | 42 (29) | 1 | 3·7 |
| III. MF of the skin with lymph node involvement | | 12 (1) | 4 (2) | 16 (3) | 3 | 1 |
| IV. MF of the skin with visceral involvement | | | 4 (0) | 4 (0) | 2 | 1 |

DLAP = dermatopathic lymphadenopathy.
* Number of patients in remission.

In Table 1 the numbers of patients per stage and the results of therapy are summarized. Table 2 summarizes the results of the different protocols for treatment.

The combined effort of the Dutch Mycosis Fungoides Study Group has lead to an increased experience in the management of patients with mycosis fungoides. However, the follow-up period is still too short to answer the question whether a more aggressive therapy in the early stages of MF changes the course of the disease.

TABLE 2. Results of different therapy regimens

| Therapy | No. of patients | No. of patients in remission |
|---|---|---|
| HN2 | 42 | 27 |
| E total skin | 26 | 21 |
| PUVA | 16 | 12 |
| Chemotherapy | 18 | 3 |

## CASE REPORTS

### Childhood dermatomyositis

A.C. DE GROOT

H.Z., female, aged 4 years.

*History*. This patient was admitted to hospital (Department of Neurology) because of progressive muscle weakness. A diagnosis of polymyositis was made. She was referred to the Department of Dermatology because of oedema of the face, and skin lesions mainly localized to the hands.

*Examination*. There is oedema of the face, mainly of the upper eyelids and cheeks, with a heliotrope colour. There is very extensive livedo reticularis, especially on the buttocks. Her lower arms and backs of the hands show some oedema. On the knees and elbows there is faint erythema with multiple small telangiectases. On the hands nearly all proximal nailfolds show ivory-coloured atrophy with telangiectases. The skin overlying several knuckles and proximal interphalangeal joints shows patches of ivory coloured atrophy with keratosis surrounded by a zone of telangiectases. Neurological examination showed signs of active polymyositis.

*Investigations*. ESR 11–30 mm; AF, SGPT, aldolase and CPK: normal. LDH 610 u/l; SGOT 50 u/l; ANF: negative. 24 h urine, creatinine-creatine index and blood gas values (venous) all normal.

*Skin biopsy*. Oedema in the upper dermis and mononuclear cells surrounding blood vessels. There is some infiltration of the vessel walls with inflammatory cells. Immunofluorescence of a skin biopsy showed continuous C3 deposition along the dermo–epidermal junction.

*Therapy*. On prednisone 20 mg daily a clinical and serological improvement was noted after some weeks. The prednisone dose was gradually reduced.

*Comment*. Early diagnosis was not a prominent feature in this case as it took 6 months before the initial diagnoses rheumatic disease and later polymyositis, were changed to dermatomyositis due to the appearance of the typical cutaneous lesions.

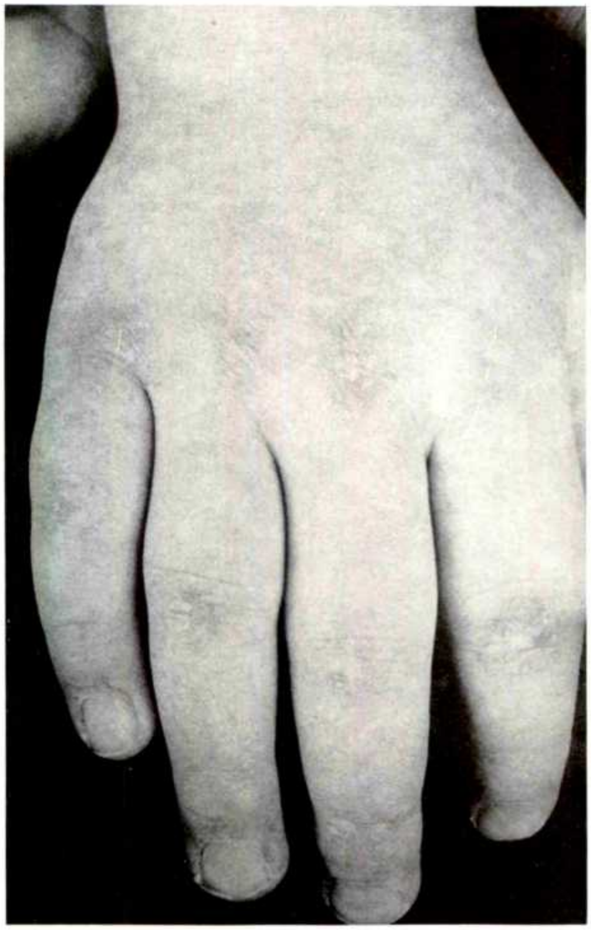

FIGURE 2. Dermatomyositis. Atrophic lesions surrounded by erythema with telangiectases. Ivory coloured atrophy with telangiectases of the proximal nailfolds.

## Chronic benign familial pemphigus (Hailey-Hailey)

L.H.H.M.DRIESSEN AND L.C.A.VAN DER HARST

G.R., male, aged 40 years.

*History*. This patient has for 6 years from time to time been troubled by an itching rash, firstly in the groins, later also on the trunk and on the neck. The lesions consist of redness, blisters and crusts. Warm weather and trauma seem to provoke his skin problem.

*Family history*. The patient's father, a brother and two uncles on his father's side of the family appear to have a similar skin condition.

*Examination*. On the trunk and on the neck there are crusted oozing erosions up to 1–2 cm in diameter. On the back also there are some flaccid blisters surrounded by a zone of erythema.

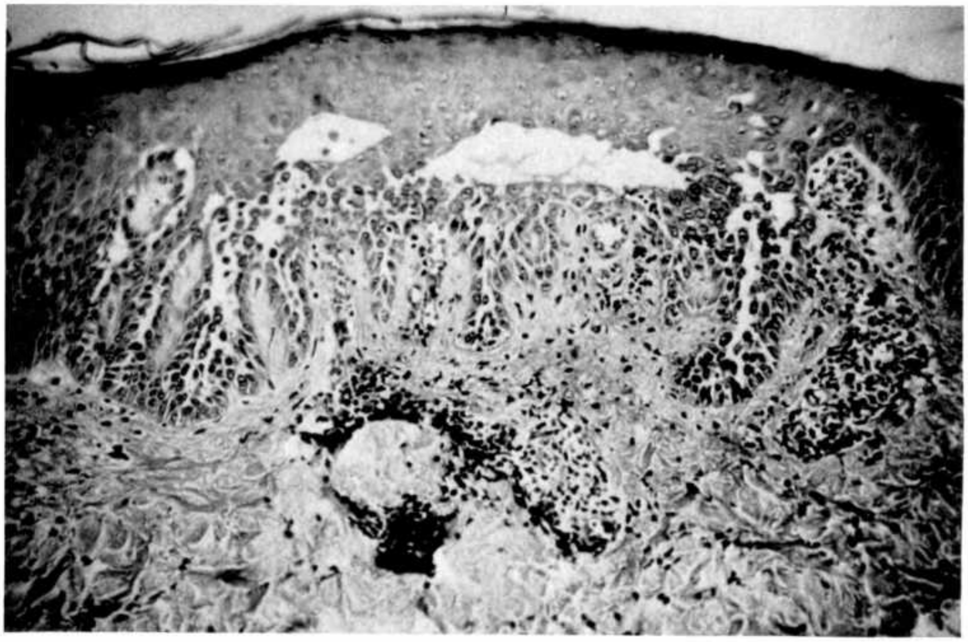

FIGURE 3. Hailey-Hailey disease. 'Dilapidated brick-wall' (haematoxylin–eosin).

*Skin biopsy*. A suprabasal intraepidermal blister. Both individual acantholytic cells and sheets of stratum spinosum cells are seen creating the appearance of a 'dilapidated brick-wall' (Fig. 3). Immunofluorescence of a skin biopsy showed no abnormalities.

*Therapy*. Local therapy has been adequate.

*Comment*. One of his relatives was elsewhere under treatment for recurrent intertrigo of the groins and only family examination provided the real diagnosis of this intertriginous disease.

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## Nail involvement in Reiter's Disease

J.W.E.DIJKSTRA

J.W.H., female, aged 74 years.

*History*. In 1976 this patient developed paronychia of all fingers and toes. Afterwards the nails became hyperkeratotic and dystrophic, and several nails showed onycholysis. Scaly lesions appeared on the extensor surface of the elbows. In 1977 she had a period of inflammation of the eye and painful micturition. During admission to the hospital in 1977 she developed inflamed and tender joints.

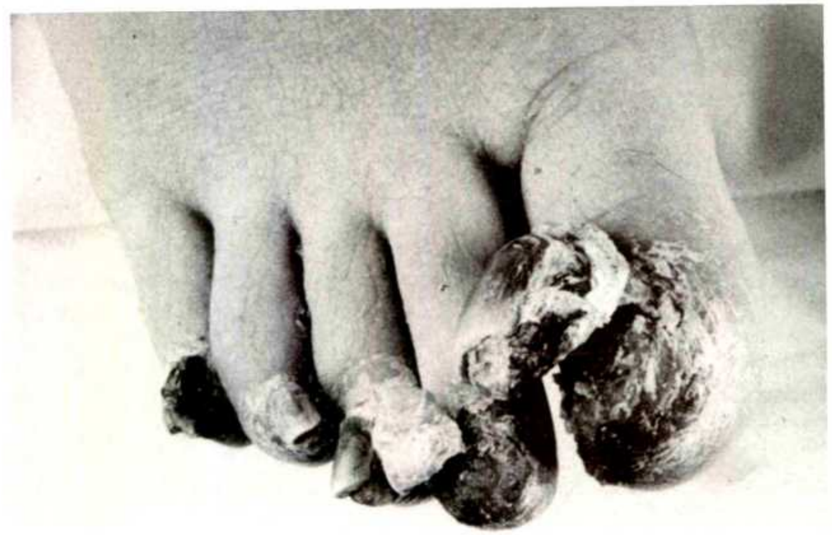

FIGURE 4. Reiter's disease. Nail changes.

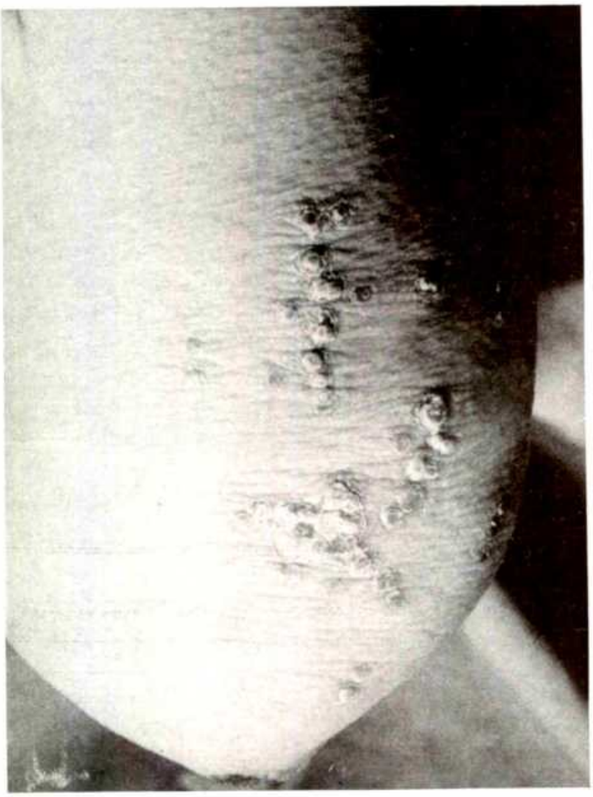

FIGURE 5. Reiter's disease. Hyperkeratotic papules on the extensor surfaces of the elbow.

*Examination.* Hands: the nails are yellow and dystrophic and show subungual hyperkeratosis. All nailfolds are red and swollen. There is diffuse palmar erythema. Feet: as described above, but no inflammation. Some nails are missing (Fig. 4). On the soles and the extensor surface of knees and elbows there are several erythematous hyperkeratotic papules (Fig. 5).

Rheumatological examination: polyarthritis of hands, wrists, elbows, knees and ankles.

Ophthalmological examination: no abnormalities.

Gynaecological examination: no urethritis.

Skin biopsy and immunofluorescence were non-informative.

*Investigations.* ESR 35 mm after 1 h; gamma glob. 31·1%; IgA 672 mg/100 ml; IgG 2993 mg/100 ml; IgM 101 mg/100 ml; AST 150; Rose Waaler negative; cryoglobulins negative; ANF-PNF negative; HLA-B 27 antigen positive. All cultures for microorganisms of nails, urethra, cervix, synovial fluid of the knees: repeatedly negative.

*Therapy.* Tetracycline 250 mg four times daily was given for 5 weeks. Nails as well as skin and joints responded promptly. After 3 months all nails were perfectly normal again.

*Comment.* In this case the rapid massive involvement of all nails and the equally rapid cure after tetracycline therapy were striking.

## Congenital non-bullous ichthyosiform erythroderma

P.J.Coenraads

M.de V., female, aged 19 years.

*History.* This patient was born as a collodion baby. After several months the skin became covered with thick scales over the entire body.

*Family history.* None of her relatives has suffered from skin disease.

*Examination.* The entire skin surface, including face and head, is covered with thick horny plaques (Fig. 6). Palms and soles show palmoplantar keratoderma. There is mild ectropion of both lower eyelids.

Neurological examination: no abnormalities.

Stomatological/dental examination: apart from extensive caries no abnormalities.

*Skin biopsy.* Showed massive orthokeratosis. Mild acanthosis with 'fingertip like' projections upwards. Many keratin cysts in the epidermis.

*Therapy.* A dramatic improvement was achieved by keratolytics, vitamin A acid 0·1% cream and oral vitamin A acid 15 mg twice daily.

*Comment.* The striking feature in this case was the fact that it proved possible in Holland to find a patient with extensive disfiguring congenital skin disease who only after 19 years asked for treatment.

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Eriksen, L. & Cormane, R.H. (1975) Oral retinoic acid as therapy for congenital ichthyosiform erythroderma. *British Journal of Dermatology*, **92**, 343.

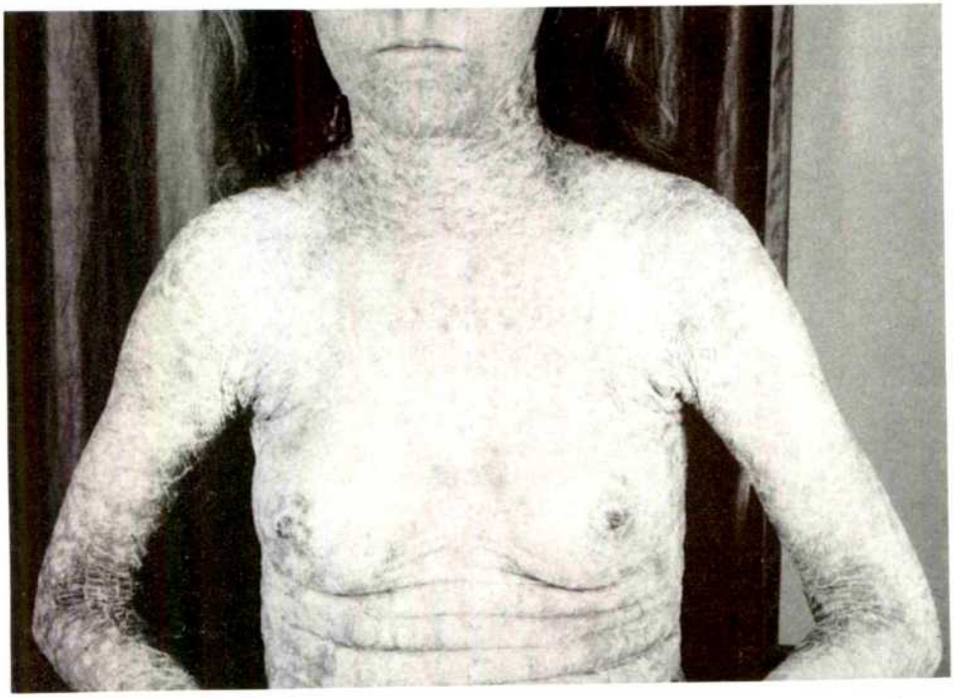

FIGURE 6. Congenital non-bullous ichthyosiform erythroderma: lamellar hyperkeratosis.

## Multiple telangiectatic glomus tumours

J.W.E.DIJKSTRA AND K.HERMSEN

A.B., male, aged 9 years.

*History*. This patient has had blue skin lesions on the back of his right upper leg, painful on palpation, since birth. The lesions seem to be growing.

*Examination*. On the back of the right upper leg there are multiple blue to purplish-blue macules, telangictases and nodules, spread over an area about the size of a palm. They are painful on pressure.

*Skin biopsy*. Dilated blood vessels in the dermis, surrounded by several layers of cells with a round or ovoid nucleus. There is no fibrotic capsule.

*Therapy*. Excision by the plastic surgeon.

*Comment*. This tumour may be less rare than it generally is considered to be, as the clinical demonstration of this case prompted one of the house surgeons to show identical lesions, which previously elsewhere had been misdiagnosed by the histopathologist as 'haemangioma'.

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MCEVOY, B.F., WALDMAN, P.M. & TYE, M.J. (1971) Multiple hamartomatous glomus tumours of the skin. *Archives of Dermatology*, **104**, 188.

## Ichthyosis linearis circumflexa

P.J.Coenraads

K.v.D., female, aged 10 years.

*History.* From the age of 6 months she has had red scaly spots, developing into semi-annular lesions (Fig. 7). Otherwise she is physically and mentally healthy.

*Family history.* None of her relatives has suffered from skin disease.

*Examination.* Trunk and extremities show semi-annular bands of erythema about 5 mm wide, covered with a fine hyperkeratosis, loosening at the edges. Suboccipitally the hairs show a fragile twisted structure. The teeth are normal.

*Investigations.* Amino acid chromatogram of urine normal; microscopy of involved hairs shows trichorrexis; intracutaneous allergy tests show positive reactions to several atopic inhalant allergens.

*Skin biopsy.* Massive parakeratosis with local crusting and absent stratum granulosum. Intercellular edema in stratum. Malpighii, at one location leading to lacuna formation.

*Therapy.* The vitamin A acid derivative, Ro 10-9359, 15–30 mg daily gave a slight improvement.

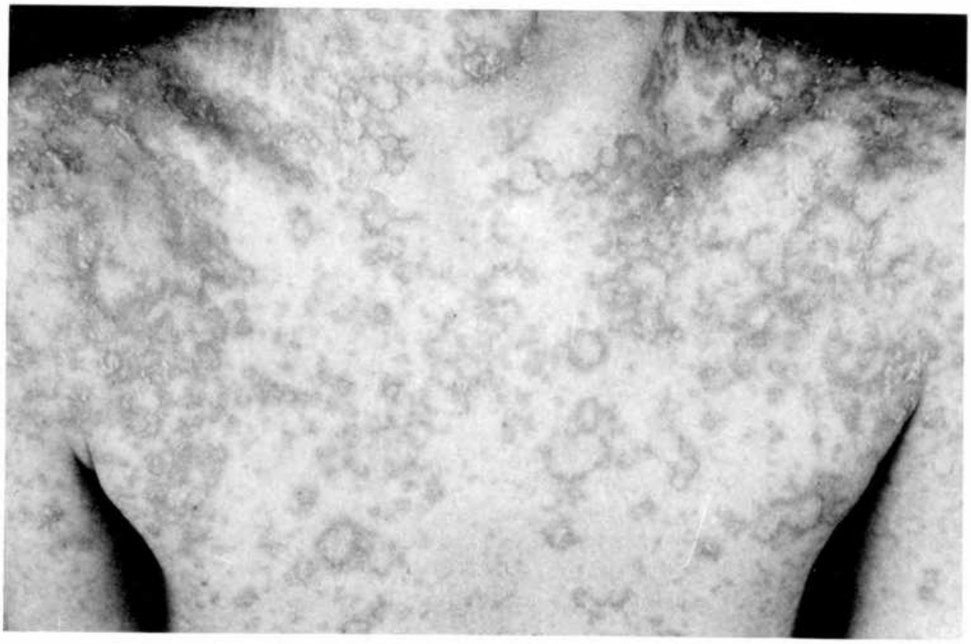

FIGURE 7. Ichthyosis linearis circumflexa.

*Comment.* Ichthyosis linearis circumflexa has been described as part of Netherton's syndrome: I.L.C., atopic constitution, amino aciduria and bamboo hairs. This patient displays a number but not all of these symptoms.

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*British Journal of Dermatology* (1980) **102,** 487.

***Review Article***

# Acute ulcerative contact dermatitis from Portland cement

Dermatologists are less familiar with acute ulcerative contact dermatitis ('cement burns') from Portland cement than they are with it causing chronic irritant contact dermatitis from wetness, grittiness and alkalinity or allergic contact dermatitis from chromate and cobalt. Since 1976, however, there has been a spate of reports of cement ulceration in the literature, which has provoked some discussion. Patch tests have always been negative. This review seeks to identify the circumstances which are necessary for Portland cement to provoke this extreme reaction.

In 1961, Rowe presented a striking case at the Fall Meeting of the Wisconsin Dermatological Society which was subsequently fully reported (Rowe & Williams, 1963). A 66-year-old man spent 2½ h kneeling in wet, ready-mixed (or pre-mixed) cement while he spread a floor. When he got up, the skin over both shins was a 'peculiar green colour'. Over the next 2 days the colour darkened and the skin over his shins and knees became so painful and haemorrhagic that he was admitted to hospital. On admission, a thick black eschar extended from 6 inches above the ankles to 3 inches above the knees on the anterior aspect of both legs. This was removed to reveal deep ulceration, and split-thickness skin grafts were applied. Both the patient's wife and neighbour were said to have worked with the same batch of cement without skin problems. The authors suggested that pressure had been a factor in the patient's severe reaction, implying that his wife and neighbour had not knelt in the cement as he had.

Vickers & Edwards (1976) reported three separate incidents of 'cement burns'. The best documented involved a 37-year-old physicist and his neighbour, a 34-year-old sales director, who ordered a delivery of ready-mixed concrete for the foundations of a new house. When the first load arrived they had to level it evenly in trenches and, finding this difficult to do with shovels, they trod it down wearing rubber Wellington boots. Both men soon had wet cement inside their boots and one of them noticed a burning sensation. By the third and last load of the afternoon the other man was also complaining of burning of the legs and feet, but they finished off the job together. That evening painful redness of the skin was noted by one man and blistering by the other. Over the next few days both men developed patchy ulceration over the feet, ankle bones, shins and calves. In one case the uppermost lesion was a horizontal ulcer corresponding to the top of the Wellington boot. In the other two incidents reported one also involved ready-mixed cement inside Wellington boots and the other a man kneeling on wet cement wearing cavalry twill trousers and then driving 200 miles without first washing off the cement. Necrosis occurred, respectively, on the dorsa of the feet and ankles and over the patellae. The authors proposed that both pressure and an abnormally high alkalinity of the cement batch had contributed to the incident involving the physicist and the sales director.

Hannuksela, Suhonen & Karvonen (1976) reported six cases of ulceration over the patellae following kneeling in wet cement for 2–6 h, and one case of ulceration on the feet following wet cement getting inside boots. Two of the patellar cases had been levelling the same floor. Smarting could be felt during the work, redness tended to be visible the same evening and necrosis to occur by the next day. The authors identified the following common features in their seven cases: prolonged exposure times,

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insufficient skin protection allowing clothes to get wet, and pressure on exposed areas. Two of the cases were in the construction industry, the remainder among amateurs.

Bandmann & Agathos (1977) reported a case of ulceration over the dorsa of the feet and shins following do-it-yourself contact with wet ready-mixed cement in a 55-year-old woman wearing shoes. The authors commented that it was the first case they had seen in 20 years experience and claimed that all reported cases had so far involved ready-mixed cement.

Jünger & Witzani (1978) reported a further case of ulceration over the patellae following kneeling for 2 h in wet cement by a 27-year-old bricklayer. He had often worked before in the same kneeling position without any trouble. They stressed the rarity of such cases as well as the sudden reaction in their patient to a situation he had been in before without reactions and discussed the aetiology at length. Special additives had not been used in the cement. An excess of free lime or gypsum was considered an unlikely explanation because this is so damaging to the structural properties of concrete that manufacturers of ready-mixed concrete rigorously control these components in their batching. Vickers & Edwards' (1976) suggestion of increased alkalinity in the batch was left as an open question.

Flowers (1978) reported another case of ulceration over the patellae and shins following kneeling in wet ready-mixed cement for about an hour. The author quoted figures for free calcium oxide in Portland cement and described this as the corrosive agent. Greening & Tonry (1978) subsequently quarreled with this author's statements about the lime content and caustic properties of Portland cement. They stressed that there is no 'free calcium oxide' in hydrated Portland cement. They stated that when concrete is fresh and plastic, calcium hydroxide is present and is the reason for 'burns' when proper protective measures are not followed. In their opinion 'it is only necessary for the worker to avoid contact on exposed skin for any prolonged length of time' (Greening & Tonry, 1978).

Fisher (1979) reported two further cases following kneeling in wet, ready mixed cement and reviewed the subject, emphasizing that warnings should be given by those who deliver or sell ready-mixed cement and that care should be taken not to allow the wet cement to make contact with the skin. Rycroft (1980) has reported a case of ulceration over the shins from wet, ready-mixed cement penetrating trousers loosely wrapped around with polythene sheeting. Wells (personal communication) has seen a case of erythema, oedema and patchy ulceration of the right hand in a tiler after tile cement had fallen inside a rubber glove he was wearing.

There appears to be fairly general agreement that direct skin contact, prolonged contact time, and pressure on exposed areas by kneeling or occlusion of cement against the skin by rubber boots, polythene sheeting or rubber gloves, are the necessary conditions for wet Portland cement to cause an acute ulcerative contact dermatitis. How are we to assess the preponderance of ready-mixed Portland cement among case reports and suggestions that alkalinity may be increased in certain batches?

The first question may perhaps be easier to answer than the second. Portland cement, ready-mixed or not, accounts for nearly 90% of all cement used in the United Kingdom (Neville, 1973). In the United States ready-mixed cement accounts for over 80% of cement usage (Ackroyd, 1962). In many countries more than half the cement used on building sites is ready-mixed (Neville, 1973). Thus the preponderance of ready-mixed, Portland cement in case reports may simply reflect that, especially for amateurs, this is the most likely type of cement to be used. It certainly does not seem to be possible to implicate any special cement additive as a common factor between all reported cases.

The main practical problem that can arise with ready-mixed Portland cement is a decrease in 'workability' with time. 'Workability' is a trade term which is difficult to define precisely, but which refers to the ease with which cement can be handled from the mixer to its final fully-compacted position (Ackroyd, 1962). Loss of workability is most rapid over the first 10 min after mixing is started but does proceed further over the next half an hour due to evaporation of water. During this time the cement is usually on its way to the site. Along with this loss of workability there is a small rise in alkalinity. Roberts (1968), for example, measured the total alkalinity of pastes of Portland cement and water. At 2 min after mixing alkalinity was approximately 0·05 N (approximate pH conversion = 12·7). There was a rapid increase in total alkalinity over the first half an hour up to about 0·07 N which then remained relatively constant for times up to 8–24 h. This increase in total alkalinity represents only one- or two-tenths of a pH unit, but such changes in pH may perhaps be significant physiologically at this borderline of irritancy.

Ready-mixed concrete as delivered to the site can, therefore, be both more difficult to spread and marginally more alkaline than freshly mixed concrete. Wide fluctuations in alkalinity from batch to batch, however, are unlikely. Ready-mixed concrete is generally made in highly automated batching plants where batching mistakes are very unlikely because they are specifically guarded against. There are two main types: central mixed and transit mixed (Neville, 1973). With the first type, mixing is done at the plant and the drum on the truck merely agitates the mixture (2–6 rev/min). With the second type, batching only is done at the plant and mixing occurs on the truck (4–16 rev/min). Usually only 70 revolutions at mixing speed are required. Maximum haulage for central mixed concrete is usually 10 miles or half an hour (Ackroyd, 1972), but distance and time can be increased by transit mixing. None of the reports in the literature to date comments on which type of mixing was used, but these details may be important to ascertain in future cases, if further light is to be shed on the aetiology of 'cement burns'.

For the present it can be said that any difficulty in spreading concrete tends to increase both the degree and duration of skin contact. If this concrete also has a marginally raised pH it is perhaps that much more likely to attack the skin under such conditions of heavy and prolonged contact. It may therefore be that the increased use of ready-mixed cement has genuinely been associated with an increased risk of acute ulcerative contact dermatitis. Alternatively the plethora of recent reports may simply represent an increased awareness of the problem among clinicians. The risk of developing this condition from cement appears to arise only if skin contact with wet cement is prolonged and combined with pressure or occlusion. There needs to be an increased awareness of this, as well as of the other potential risks of cement to the skin, among both amateur and professional cement users.

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*British Journal of Dermatology* (1980) **102**, 491.

## *Obituary*

### DR ALICE CARLETON

Alice Carleton died peacefully in the early hours of 1 December 1979. Thus ended the life of one who had become a legend in her life-time.

She was born in Dublin on 26 September 1891, the fourth child and first daughter of Sir Arthur Chance, a President of the Royal College of Surgeons of Ireland. Although her mother died at her birth, after her father's second marriage, she became one of a large family and had the great experience of growing up in the centre of Edwardian Dublin Society.

She was educated in Dublin at a Dominican Convent School and went to Oxford in 1909 to study English Literature, living in St Frideswide's Convent. She took a degree in English with honours in the Dublin National University in 1911 but she often used to say that when her father saw the content of some of her books he thought that she would be better employed reading Medicine. This she did, qualifying, again with honours, in Dublin in 1917, going on to take the M.D. in 1944.

Soon after qualifying, she was invited by Professor Arthur Thomson to join the Medical School in Oxford to teach anatomy to the small number of female medical students. However with the influx of students after the Great War, the majority of whom had been on Active Service, this sex discrimination lapsed and she became a fully integrated member of the anatomy department. It was soon recognized that she was a born teacher and not only gave the routine lectures and demonstrations but she was in great demand as a coach. The stories about her are legion, some almost certainly apocryphal, but there is no doubt that she had an enormous influence on many generations of Oxford medical students. I have been approached in many parts of the world by distinguished holders of the Oxford medical degree whose first question invariably has been 'How is Alice?'.

She became interested in the skin and its diseases in the 1920s and helped Dr Ernest Mallam, a General Practitioner who was in charge of the skin department of the Radcliffe Infirmary. He was President of the BAD in 1928 and Alice Carleton appears on the group photograph taken at that meeting. In 1929 she was appointed Assistant Physician to the skin department and spent time studying in Paris and Vienna. She took charge of the department in 1938 and in spite of her very busy hospital clinics and private practice, she still remained an active teacher in anatomy until she retired in 1957. After the War she was invited to open skin clinics at Banbury, Swindon, Marlborough and Cirencester and she started the regular services in each of these hospitals. By the time she retired, the amount of work had become so great that Swindon, Marlborough and Cirencester were separated from Oxford and appointed their own dermatologist.

In spite of this pressure of work, she published frequently and was also a very regular attender at the early foreign excursions of the Dowling Club where her zest for life and gift of tongues were tremendous assets. She was also a very welcome visitor to many of the European clinics. She was President of the BAD in 1951, a meeting which the older members will remember with pleasure.

She retired from the United Oxford Hospitals in 1957, her last day being marked by a ceremonial procession based on the theme Alice leaves Wonderland. She then spent a year as visiting lecturer in anatomy at the University of California, subsequently she held a similar position in Yale and she thoroughly enjoyed teaching the more mature and more outspoken American medical students. When she returned to this country, she left her hospitable home in Banbury Road, Oxford and settled in a house in Haddenham where she had a love-hate relationship with her devoted housekeeper who predeceased her. She worked hard in her garden and since whatever she did, she did well, the garden was featured in one of the glossy magazines.

She married Harry Carleton, an outstanding histologist, in 1923 and although this marriage was dissolved in 1940, they remained friends until his sudden death in 1957; indeed he washed her car regularly on Saturday mornings. She lived a very active life in retirement, was a formidable bridge player and indulged her love of travel to exotic places, climbing the Mexican pyramids when over the age of 70, relishing an African safari and trips to the Far East. She enjoyed meeting people and was a very welcome guest at the last Oxford BAD meeting.

In the last few years of her life, although still looking much the same without a hair—sometimes slightly blue—out of place, she realised that she was losing her memory and moved to an attractive Home situated on the riverside at Goring. With increasing age she could not see quite so well and although conversing lucidly tended to ask the same questions repeatedly but in spite of this was able still to sparkle in her own inimitable manner. She became stuporons about 10 days before she died, sustained by the deep faith which she had kept all her life. She was buried at St Philip's Priory, Begbroke in a position which she told me she had chosen herself.

All who were privileged to know her are so much the better for her stimulus. A unique, modest, dynamic lady who has a recognized place among the dermatological giants of the previous generation, Dowling, Barber, Gray, Hallam, Ingram, McCormac and others.

RENWICK VICKERS

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# *Book Reviews*

## Operative Surgery. Plastic Surgery.

3rd edn. J.WATSON and R.M.MCCORMACK (1979) Sevenoaks, Kent: Butterworth. Pp. 556, B/W illustrations. Price £55.00.

'Fundamental international techniques' is the subtitle of this book, and as such the editors with their international panel of contributors have adhered to the techniques of plastic surgery. The techniques described may be fundamental to the established plastic surgeon, but they are certainly not part of the stock in trade of the dermatologist. There is no mention of the common methods used in skin surgery for the treatment of the minor skin lesion, and excision appears to be the only method suggested for the treatment of a basal cell epithelioma. Apart from reconstruction of the breast and rhinoplasty there is little mention of cosmetic surgery. This is not an atlas of plastic surgery which is going to inform the interested spectator what plastic surgery can do to help those problems which confront the dermatologist, nor is it going to help the dermatologist deal more expertly with the skin problems which are commonly met in out-patients. Plastic surgeons appear to focus in larger texts on the major aspects of their specialities so that there are lengthy chapters on skin flaps of all sorts and sizes, on musculocutaneous flaps now constructed as free flaps with microvascular anastomoses, on complex facial reconstruction and on repairs of eyelid or nose. Many of these procedures are carried out for extensive or recurrent skin cancers, such that it is hard to avoid the thought that if only the first procedure had been adequately performed these complex procedures might not have been necessary. However, the great skill and the recent advances in plastic surgery must not be underestimated. If only these talents could be better communicated, there would be great advantage to all. Unfortunately, this book will not help communicate that skill to others, as it is a book for the specialist, who already understands much of the techniques of plastic surgery, and is not for a beginner who is trying to apply these techniques in other specialities.

CHRISTOPHER RUSSELL

## Modern Topics in Paediatric Dermatology.

Edited by JULIAN VERBOV (1979) London: William Heinemann Medical. Pp. 220. Price £11.50.

This book is a series of essays on paediatric aspects of dermatology by various authors. The common disorders are included as well as some rather more rare, such as immunodeficiency and psychosomatic aspects of skin disease. Unless there is very strict editing, this format can produce unnecessary repetition of some subjects and omission of others and this book is no exception. Some topics such as napkin psoriasis, Ehlers-Danlos and Wiskott-Aldrich syndrome receive repeated attention whereas histiocytosis X and common conditions such as contact dermatitis, are omitted. There is, however, a high degree of continuity of lay-out and style in each section. The photographs are all black and white and the quality of reproduction of some is such that they could readily have been omitted without loss. With the authors invited by the editor to write for this book one would expect, and does find, an authoritative, informative and readable package.

It is said to be specially aimed at paediatricians, family practitioners, school medical officers as well as dermatologists. I think it would be impossible for any book to have a strong appeal to such a wide range. It would probably find greatest acceptance to paediatricians with this format and content.

D.BURROWS

## Your Skin. Its Problems and Care.

SETRAG A.ZACARIAN (1979) Tunbridge Wells: Abacus Press. Pp. 163. Price £5.50.

Shorn of its American idiom and, even more to the point, of its basis in American dermatological practice (from which, for example, stem parochial details of how to find a dermatologist direct, where we would consider the general practitioner; references to the F.D.A. and proprietary names; and a full account of the venereal diseases), this book would be useful. Some patients would welcome further information on their own skin problems or on those of their friends and relations.

The main topics covered are birth marks, acne, psoriasis, venereal diseases, sun-mediated effects, cancer, cosmetics, hair, ageing changes and warts. The accounts are flanked by an introductory chapter on structure and function, and a concluding one on assorted lesions which is followed by a note on recent advances, questions and answers, a glossary and an index. The style and tone are pleasant and the matter sufficiently sound.

C.M.RIDLEY

*British Journal of Dermatology* (1980) **102,** 495.

# *News and Notices*

## International Conference of Paediatric Dermatology, Zagazig, Egypt

The First International Conference of Paediatric Dermatology will be held in Zagazig, Egypt, 21–24 June 1980.

Topics include infectious skin diseases, nutritional skin disorders, immunological skin abnormalities and other related subjects in infancy.

Further information may be obtained from The General Secretary, Professor Mohamed Amer, Chairman of Dermatology and Venereology Department, Zagazig University, Zagazig, Egypt.

## Dermatopathology Workshop, Worcester, Massachusetts

A 2-day Dermatopathology Workshop will be held on Monday and Tuesday, 25–26 August 1980 at the University of Massachusetts Medical School in Worcester. The programme will include study of 100 unknown microscopic slides of inflammatory dermatoses and skin tumours with accompanying clinical histories and multiple choice answers. The cases will be discussed by a faculty that includes Drs Jag Bhawan, Martin Brownstein and Kurt Stenn. The course is sponsored by the Office of Continuing Medical Education and Department of Pathology, University of Massachusetts Medical School, Worcester, Massachusetts and co-sponsored by the American Society of Dermatopathology. For further information contact: Dr Jag Bhawan, Course Director, Dermatopathology Workshop, University of Massachusetts Medical School, Worcester, Massachusetts 01605, U.S.A. Tel: 617 (856-2412).

## Bill Reed International Travelling Fellowships

Three fellowships will be awarded this year as follows:

(1) The winner of the Poster Session at the annual meeting of the European Society for Dermatological Research will be awarded $800.00 to help support a trip to attend the annual meeting of the American Academy of Dermatology.

(2) The winner of the Stelwagon Prize for the best paper presented at the Resident's Forum at the annual American Academy of Dermatology meeting will receive an $800.00 award to support a trip to Europe where the awardee will present a paper at the annual meeting of the British Association of Dermatologists in England. Further travel to European centres can also be arranged.

(3) A third award of $800 00 will be given to an academically oriented dermatologist in the Private Practice of Clinical Dermatology, who has been out of training no more than 5 years Anyone interested in applying for this award should send their curriculum vitae, and a covering letter indicating how they plan to spend the award funds, to the Chairman of the Committee on International Affairs of the A.A.D., 820 Davis Street, Evanston, Illinois 60201, U.S.A. The deadline for submission is 1 September 1980.

## Appointment

### Sheffield Area Health Authority (Teaching)

Christine I. Harrington has been appointed to the post of Consultant Dermatologist at the Royal Hallamshire Hospital, Sheffield. She graduated M.B.Ch.B. Hons(Sheffield) in 1971. Prior to specializing in dermatology, she obtained wide experience in acute general medicine in the Sheffield hospitals. In 1974 she became a member of the Royal College of Physicians. In 1975 she took up her dermatology training in the Rupert Hallam Department of Dermatology in Sheffield Dr Harrington has done extensive research into bullous disorders and was awarded her M.D., with commendation, on 'Dermatitis Herptiformis' in 1978.

She has published work on clinical and investigative dermatology. Her main research interests are the immunopathology of bullous eruptions and cutaneous lymphomas.

# NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

**Manuscripts** should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$ g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$ g) | µg | cubic millimetre(s) | $mm^3$ | micrometre(s) | µm |
| nanogram(s) ($10^{-9}$ g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$ g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}I$ |
| hour(s) | h | milliequivalent | mmol | | |

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

**Illustrations** should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

**References.** Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

**Page proofs** will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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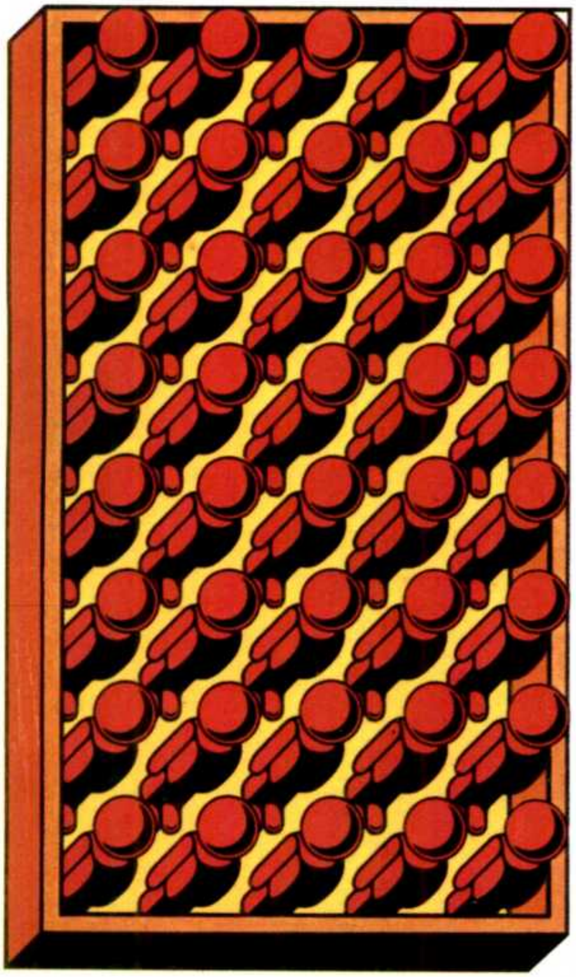
Printed in Great Britain at the Alden Press, Oxford

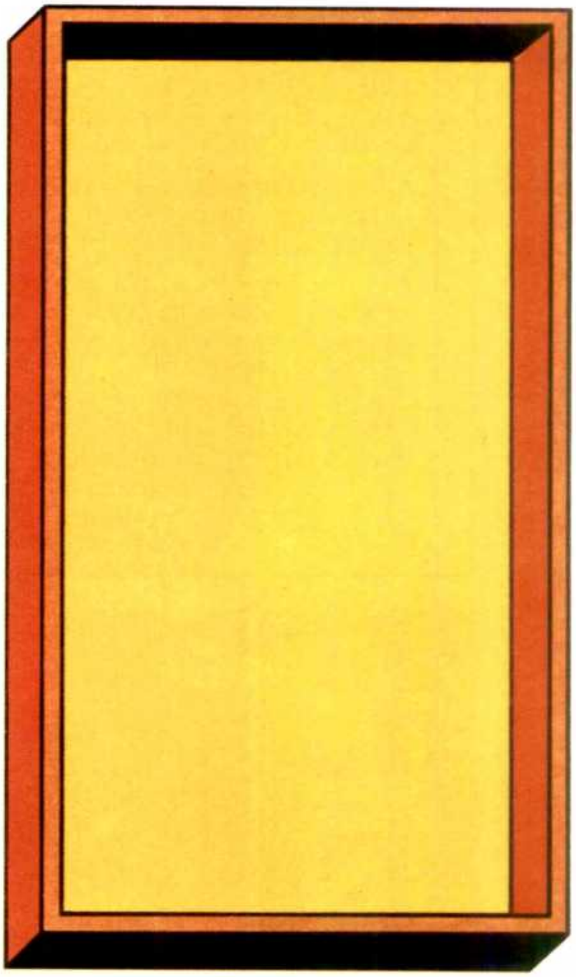
# Dithrocream

dithranol BP

# Natural selection!



**All 40 patients in the trial preferred Dithrocream**



**Not a single patient preferred dithranol ointment**

In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

Whilst both preparations were effective, there was a unanimous patient preference for Dithrocream with all 40 patients selecting the vanishing cream formulation.

Combining effectiveness, convenience and stability, Dithrocream is the patient's choice for psoriasis treatment.

**Presentation**
Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.
Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

**Indications**
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

**Directions for use**
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

**Contraindications**
Acute psoriasis; dithranol sensitivity.

**Precautions**
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

**Basic N.H.S. prices:**
Dithrocream 0.1% 50gm tubes £1.81
Dithrocream 0.25% 50gm tubes £1.96
Dithrocream Forte 50gm tubes £2.19

**Product Licence Nos.**

| | |
|---|---|
| Dithrocream 0.1% | PL 0173/0029 |
| Dithrocream 0.25% | PL 0173/0028 |
| Dithrocream Forte (0.5%) | PL 0173/0027 |

**References:**
1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
Dermal Laboratories Limited,
Gosmore, Hitchin, Hertfordshire SG4 7QR.

Dithrocream is a registered trade mark.

**Now available in three strengths**

# PUVA for Psoriasis

PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position. There are also Waldmann PUVA 180, 200 and 500 units for the treatment of hands, feet and other small areas.

A regime has been developed based on a London Teaching Hospital's experience and practice. The staff are professionally qualified and trained in the Photochemotherapy of psoriasis. Visits by Dermatologists are welcome by appointment.

An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

The Physician-in-Charge,
Psoriasis UVA (Medical Referals Only) Ltd.,
20 Harley Street, LONDON W.1.
Telephone 01-580 0875

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-for infected leg ulcers and pressure sores

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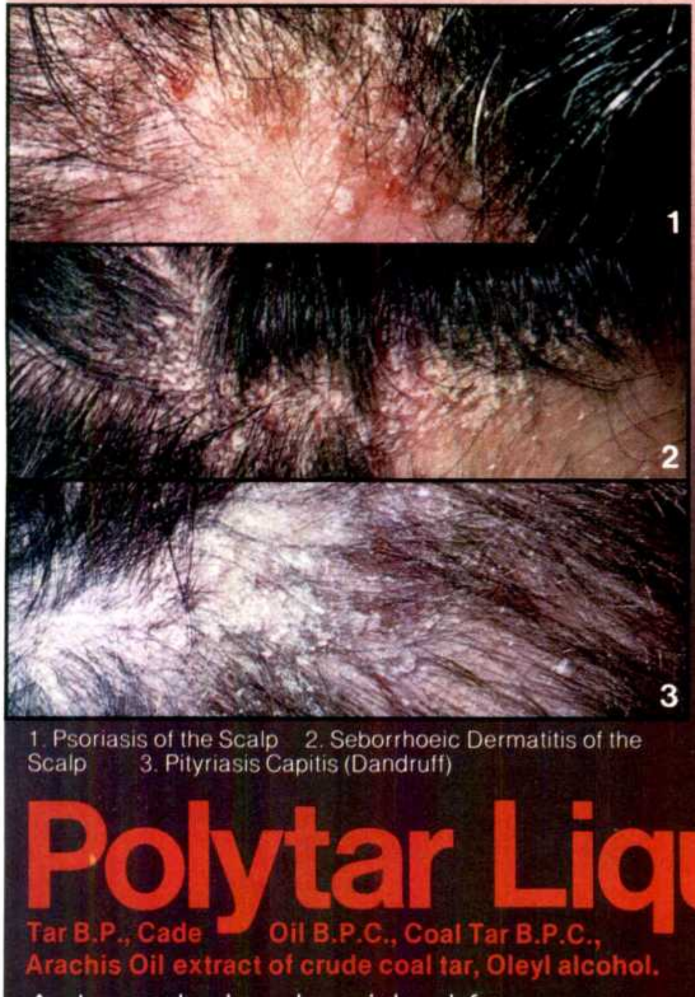

# Common Disorders of the Scalp.

They all have one thing in common — the treatment

1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

## Polytar Liquid

**Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.**

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

### Prescribing Information.

**Presentation** Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

| | |
|---|---|
| Tar B.P. | 0.3% |
| Cade Oil B.P.C. | 0.3% |
| Coal Tar B.P.C. | 0.1% |
| Arachis Oil extract of crude coal tar | 0.3% |
| Oleyl alcohol | 1.00% |

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

**Uses** Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

**Dosage and Administration** The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

**Contra-indications, warnings, etc.** Nil

**Pharmaceutical Precautions** Store in a cool place

**Packs and Prices**

| | Pack | Basic NHS price |
|---|---|---|
| Polytar Liquid | 65ml | 57p |
| | 150ml | 82p |
| | 350ml | £1.48 |
| | 1 litre | £3.90 |
| Polytar Plus | 150ml | 90p |

**Product Licence Number**
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ

# ACNE RESPONDS TO QUINODERM

## Effective treatment at an economical price

**BRITAIN'S LEADING ACNE RANGE**

Reference:
"The best topical agents are probably the benzoyl peroxide containing group which are anti-bacterial and peeling agents".
*"Focus — Dermatology" Medical News, Dec 14th issue, Suppl.*

**The QUINODERM range is now available in three presentations to meet individual prescribers needs. All contain benzoyl peroxide plus potassium hydroxyquinoline sulphate, a specific broad spectrum anti-bacterial. They are highly acceptable cosmetically and combine proven effectiveness with competitive pricing.**

**QUINODERM LOTIO-GEL**
is the latest addition to the range. This new pharmaceutical form, with its unique delivery system, has been specifically designed for those who prefer either a lotion or a gel. It is presented in a compact plastic bottle containing 30ml., which can be conveniently carried at all times in a handbag or the pocket (Basic N.H.S. cost—87p).

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is the original British benzoyl peroxide formulation. Regular and continued usage of this proven medication maintains overall control, reducing the risk of unpleasant residual scarring. Formulated as an astringent cream base, this preparation is available in tubes of 25g. (Basic N.H.S. cost—60p).

**QUINODERM CREAM WITH 1% HYDROCORTISONE (30g.)**
is also available and suggested when a one pack initial treatment with a specific anti-inflammatory response is required (Basic N.H.S. cost—95p).

---

**PRESCRIBING INFORMATION**

**QUINODERM LOTIO-GEL,
QUINODERM CREAM**

*Active Ingredients:*
Benzoyl peroxide 10%, Potassium hydroxyquinoline sulphate B.P.C. 0.5%.
*Indications:*
Acne Vulgaris and Acneform Eruptions.
*Dosage:*
By gentle massage over all the affected area, two or three times daily.
*Contraindications, Warnings etc:*
Patients with known sensitivity to either of the active constituents. In a few isolated cases overreaction to the preparation may occur. To minimise this possibility, select a small area of skin behind the ear, apply the preparation and leave for twelve hours. If severe irritation or pronounced redness occurs do not proceed.
Contact with mouth and eyes should be avoided.
Care should be taken to avoid contact with dyed fabrics as this preparation may adversely affect dye fastness.
P.L. No: **Quinoderm Lotio-Gel 0291/0007**
**Quinoderm Cream 0291/5000**

**QUINODERM CREAM
WITH Hydrocortisone**

*Active Ingredients:*
Benzoyl peroxide 10%. Potassium hydroxyquinoline sulphate B.P.C. 0.5%, Hydrocortisone B.P. 1%.
*Indications:*
Severe Acne Vulgaris, Acne Rosacea and Acneform Eruptions.
*Dosage:*
By gentle massage over all the affected area, two or three times daily.
*Contraindications, Warnings etc:*
As Quinoderm Cream and Quinoderm Lotio-Gel.
In addition, topical steroids should not be used extensively in pregnancy i.e. in large amounts for long periods.
In infants, long-term continuous topical therapy should be avoided. Adrenal suppression can occur even without occlusion.
P.L. No: **0291/5001**

QUINODERM LTD., MANCHESTER ROAD, OLDHAM, OL8 4PB

# Difficult Dermatoses

# Dermovate

(**clobetasol propionate**)

Even long-standing, stubborn eczematous lesions respond quickly to Dermovate – with rapid relief from itching; and this is often followed by prolonged periods of remission. And because Dermovate is so rapidly effective, treatment need be of short duration only, minimising the risk of steroid side effects.

**Prescribing information**

**Dosage and administration**
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

**Side effects**
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.
This is more likely to occur in infants and children, and with occlusion. In infants, the napkin Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

**Precautions**
Long-term continuous therapy should be avoided, particularly in adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.
Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

**Contra-indications**
Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines. Viral diseases of the skin, e.g.,

**Basic NHS cost (exclusive of VAT)**
Dermovate Cream or Ointment 25 gram tubes £1·74 (also available in 100 gram tubes).

| **Presentation** | **Product Licence number** |
|---|---|
| Dermovate Cream | 4/0219 |
| Ointment | 4/0220 |

***Glaxo***
**Leaders in topical steroid therapy**

Further information on Dermovate (trade mark) is available from:
Glaxo Laboratories Ltd

**Another Munksgaard Journal**

# Journal of Cutaneous Pathology

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*Journal of Cutaneous Pathology* publishes manuscripts of the highest scientific quality in the field of cutaneous pathology and aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology is interpreted to mean diseases of the epidermis, dermis and cutaneous appendages. Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication. Manuscripts based on light and electron microscopy, histochemistry, microbiology, biochemistry, immunology, pharmacology, tissue culture, embryology and genetics are welcome, provided they advance the science of cutaneous pathology.

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**INTERNATIONAL PUBLISHERS LTD.**

**35 NÖRRE SÖGADE P.O. BOX 2148 DK-1016 COPENHAGEN K DENMARK**

# When efficacy is the need and safety the concern

No need now to sacrifice efficacy for safety because Eumovate fulfils the need for a corticosteroid preparation with greater topical activity than hydrocortisone, yet with a wide margin of safety.

**Prescribing information**

**Uses**

Eumovate is suitable for treating the mi forms of eczema, seborrhoeic dermatit and other steroid responsive skin conditions.

**Dosage and administration**

Apply up to four times a day until improvement occurs, when the frequen of application may be reduced.

**Contra-indications**

Bacterial, fungal or viral diseases of the skin.

**Precautions**

In infants and children, long-term continuous topical corticosteroid therap should be avoided where possible, as adrenal suppression can occur. In infan the napkin may act as an occlusive dressing, and increase absorption. The least potent corticosteroid which will control the disease should be selected. Appropriate antimicrobial therapy shou be used concurrently whenever treating inflammatory lesions which have becom infected.

Any spread of infection requires withdrawal of topical corticosteroid therapy and, possibly, commencement systemic chemotherapy.

As with all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

**Side effects**

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately. If large areas were to be treated, some patients could absorb sufficient steroid to cause transi adrenal depression despite the low degr of systemic activity associated with clobetasone butyrate.

Local atrophic changes could occur wh moisture increases absorption of steroi but only after prolonged use.

**Basic NHS cost** (exclusive of VAT) 25 gram tube £1·48 (also available in 100 gram tubes).

**Product Licence numbers**

Cream: 4/0233

Ointment: 4/0254

**Glaxo**

**leaders in topical steroid ther**

Further information on Eumovate (trad mark) is available from:

Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

# Eumovate

**(clobetasone butyrate)**

**a unique balance of efficacy and safety**

**Another Munksgaard Journal**

Contact
Dermatitis

Environmental and Occupational Dermatitis

**Editor**
C. D. CALNAN, M.D., London

*Contact Dermatitis* is designed primarily as a journal for clinicians who are interested in various aspects of environmental dermatitis. This includes both allergic and irritant (toxic) types of contact dermatitis, occupational (industrial) dermatitis and consumers' dermatitis from such products as cosmetics and toiletries.
The journal aims at promoting and maintaining communication among dermatologists, industrial physicians, allergists and clinical immunologists, as well as chemists and research workers involved in industry and the production of consumer goods.

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# Peo le with pso i sis tend to hide themselves

It's not just the unsightly lesions. It's also the sheer discouragement, even despair. The available treatments seem to be either inadequate or cosmetically repugnant.

Alphosyl HC gives the psoriatic – and her doctor – grounds for hope. It is a cosmetically acceptable cream containing coal tar to slow down the psoriatic process, hydrocortisone in low dosage for a controlled anti-inflammatory effect, and allantoin to soften psoriatic plaques and aid in their removal.

A published clinical study* has shown Alphosyl HC to be broadly comparable in efficacy to betamethasone, and somewhat superior in its descaling action.
And Alphosyl HC is preferable on safety grounds since it does not contain a fluorinated steroid.

# Alphosyl HC

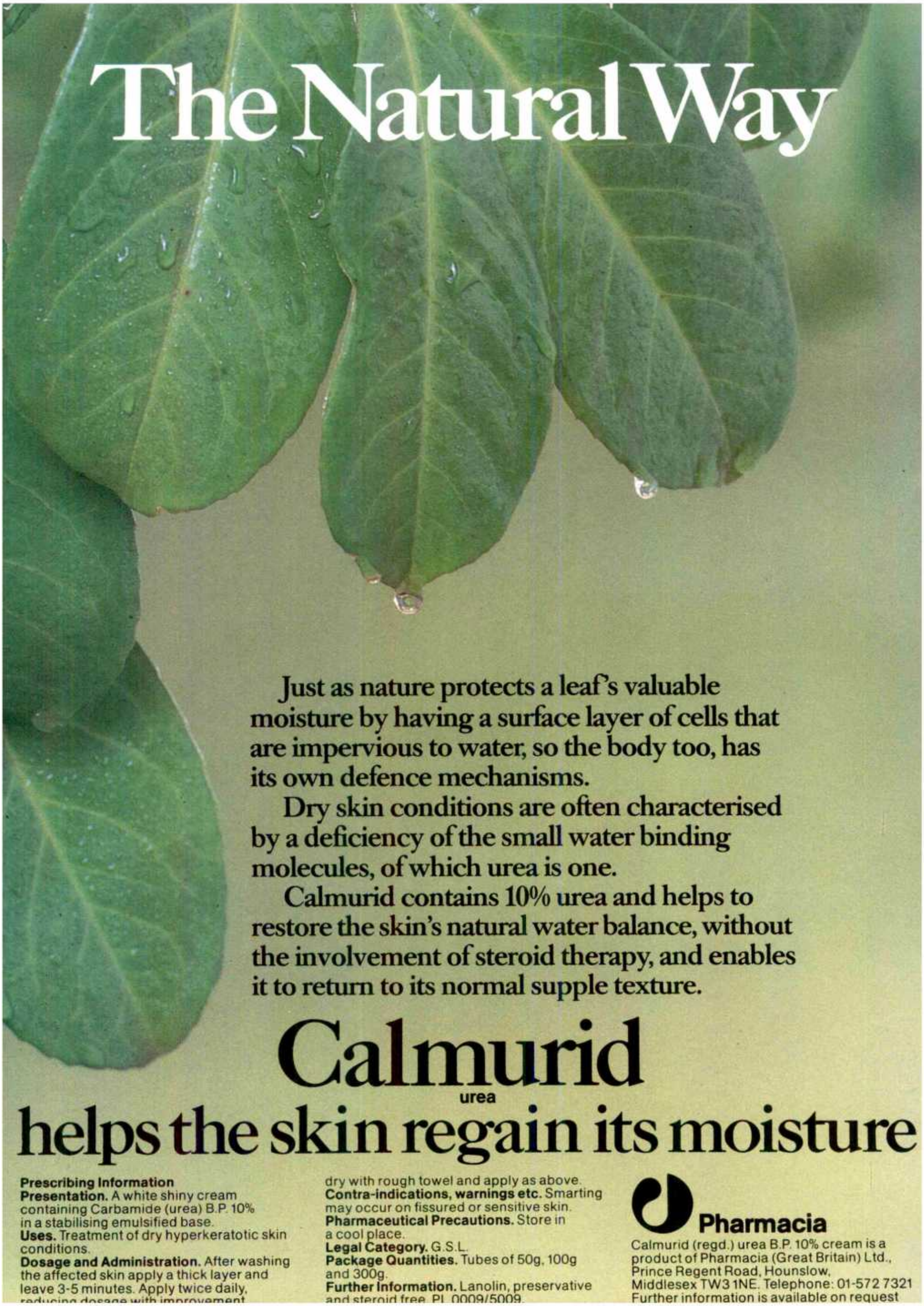
hydrocortisone 0.5% with allantoin and coal tar extract.

**purpose-designed for psoriasis**

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol. 33 No. 4, 106.

**Presentation** A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, u.s.p. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and Administration** Apply two to four times daily massaging vigorously into the affected areas. **Contra-indications and Warnings, etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of c[illegible]costeroids to pregnant animals can cause abno[illegible]ality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tubes of 30gm and 45gm. **Basic NHS cost** £1.62 for 30gm, £2.29 for 45gm. **Product Licence No.** 0036/0026

Further information is available from
**Stafford Miller Limited**, Professional Relations Division, Hatfield, Herts. AL10 0NZ



# The Natural Way

Just as nature protects a leaf's valuable moisture by having a surface layer of cells that are impervious to water, so the body too, has its own defence mechanisms.

Dry skin conditions are often characterised by a deficiency of the small water binding molecules, of which urea is one.

Calmurid contains 10% urea and helps to restore the skin's natural water balance, without the involvement of steroid therapy, and enables it to return to its normal supple texture.

# Calmurid
urea
# helps the skin regain its moisture

**Prescribing Information**
**Presentation.** A white shiny cream containing Carbamide (urea) B.P. 10% in a stabilising emulsified base.
**Uses.** Treatment of dry hyperkeratotic skin conditions.
**Dosage and Administration.** After washing the affected skin apply a thick layer and leave 3-5 minutes. Apply twice daily, reducing dosage with improvement. dry with rough towel and apply as above.
**Contra-indications, warnings etc.** Smarting may occur on fissured or sensitive skin.
**Pharmaceutical Precautions.** Store in a cool place.
**Legal Category.** G.S.L.
**Package Quantities.** Tubes of 50g, 100g and 300g.
**Further Information.** Lanolin, preservative and steroid free. PL 0009/5009.

**Pharmacia**

Calmurid (regd.) urea B.P. 10% cream is a product of Pharmacia (Great Britain) Ltd., Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321
Further information is available on request

# Why mess about with topical antifungals

# ...when oral Grisovin can be working from within?

For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

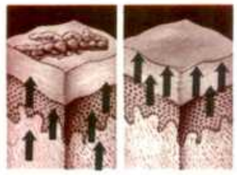
Grisovin is carried in the bloodstream to the basal layer and gets right to the root of ringworm infections. It protects the newly formed keratin cells from infection and as they move outwards, the fungus is expelled.

During the past 20 years, Grisovin has proved itself in clinical use to be the more effective therapy especially in long established ringworm infections. In addition its oral administration, two 500mg tablets a day, and its relative lack of side effects facilitates patient compliance.

Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

## GRISOVIN

griseofulvin



## THE INSIDE STORY ON FUNGAL INFECTION

### Prescribing information

**Dosage and administration**

Doses should be taken after meals for maximum absorption.

*Adults:* normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

*Children:* usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

*Duration of treatment:* for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

**Contra-indications**

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

**Precautions**

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

**Side effects**

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

**Further information**

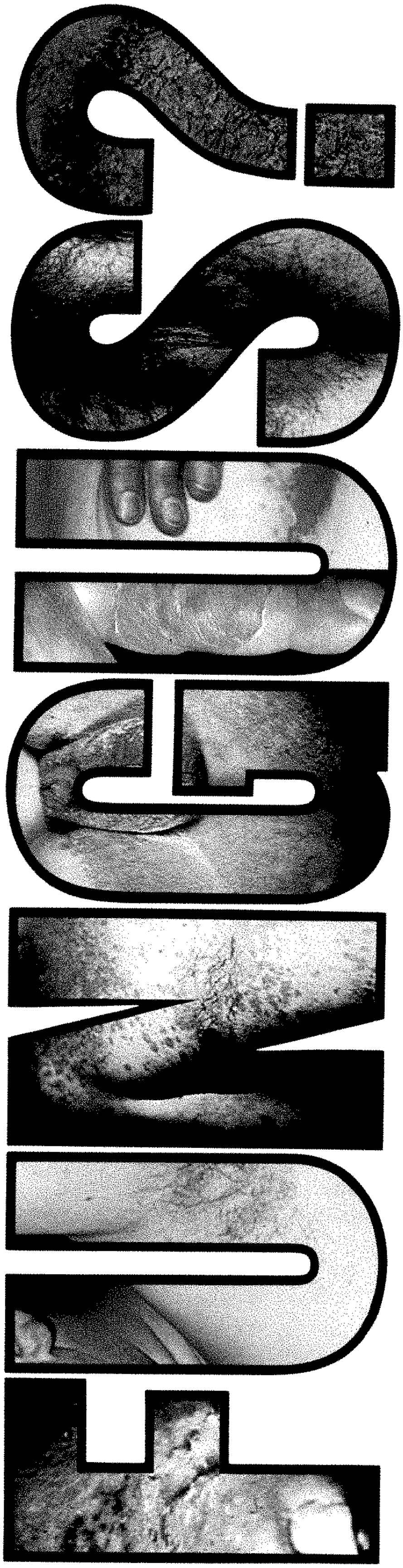
Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

| **Basic NHS cost** | **Product Licence numbers** |
|---|---|
| (exclusive of VAT) £8·91 | |
| Bottle of | 125mg 4/5060 |
| 100 tablets (500mg) | 500mg 4/5061 |

**Glaxo**

Full prescribing information on Grisovin (trade mark) is available from:

Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE



# Canesten®

clotrimazole

## Effective against all fungal skin infections

So effective treatment with Canesten may begin at once – without waiting for culture results.

Wherever on the body a fungal infection occurs, whatever the organism responsible, there is a need for speedy effective therapy. Canesten's broad range of presentations is the answer... cream, atomiser spray and now Canesten powder for adjunctive therapy and to help prevent re-infection.

## Canesten
### the most widely-prescribed antifungal

**Prescribing Information**

**Presentations** *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder containing 1.0% clotrimazole.

**Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections.

**Dosage and Administration** *Cream, Atomiser Spray* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection.

**Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation.

**Precautions** Canesten atomiser spray should not be used near a naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled.

**Product Licence Numbers** Cream: PL 0010/0016. Atomiser Spray: PL 0010/0060. Powder: PL 0010/0067.

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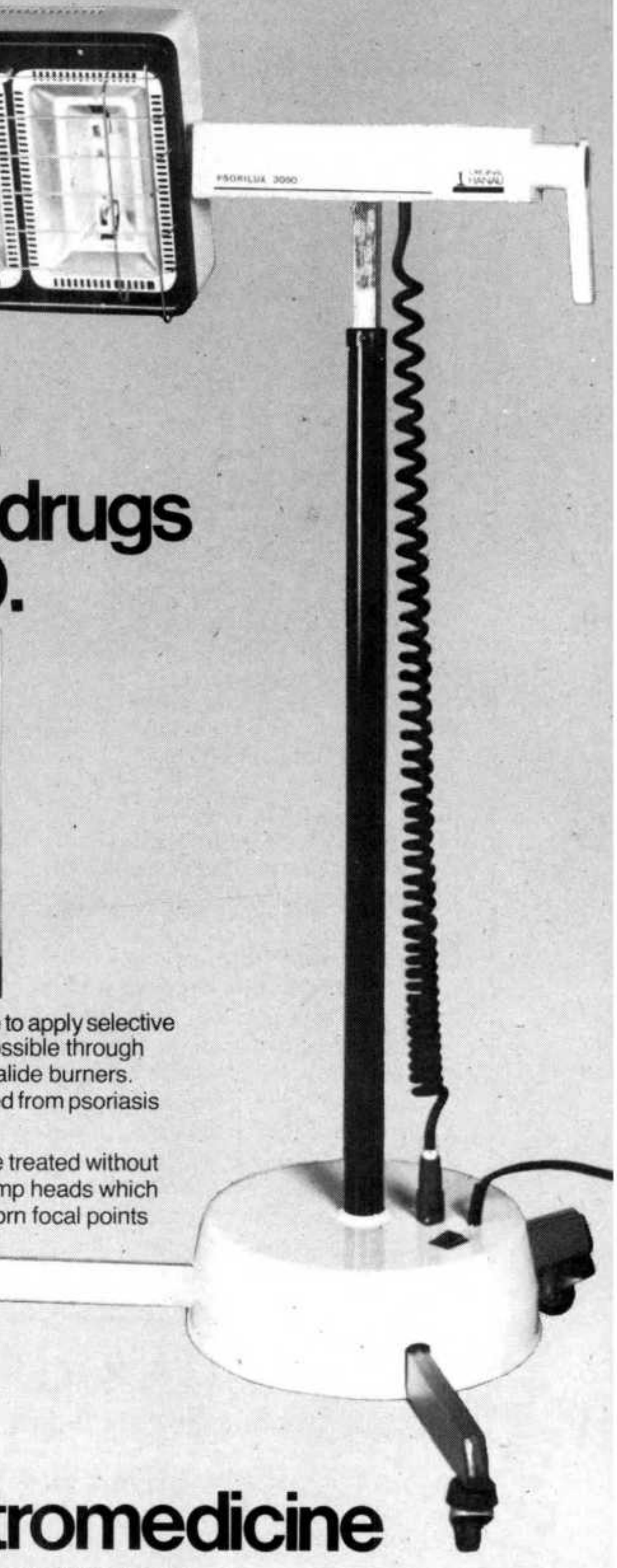

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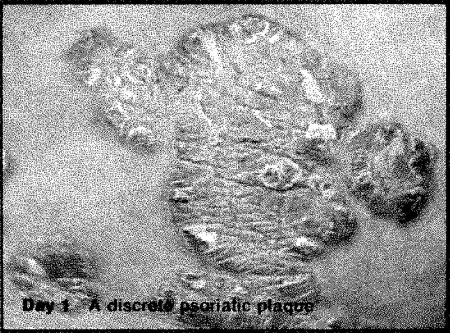
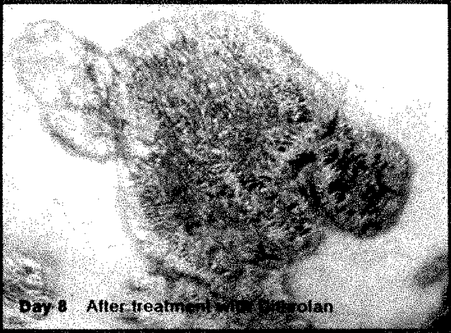
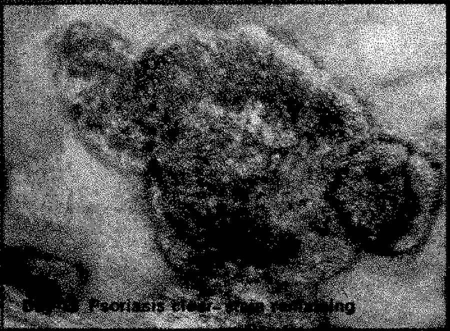
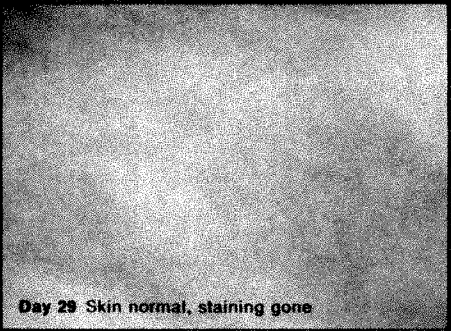

Day 1 A discrete psoriatic plaque


Day 8 After treatment with Dithrolan


Day [illegible] Psoriasis clear - stain remaining


Day 29 Skin normal, staining gone

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*British Journal of Dermatology* (1980) **102,** 497.

***Clinical and Laboratory Investigations***

# Serum proteins in human squamous epithelium

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Accepted for publication 20 August 1979

## SUMMARY

A new immunoperoxidase staining technique of high specificity (the labelled antigen method) has been used for the detection of serum proteins in the epithelium of human skin and mucous membranes. The majority of specimens contain cells showing strong diffuse intracellular staining. These cells usually lie in the upper mid-zone of the epithelium and are often characterized by vacuolated or pyknotic nuclei. Double immunoenzymatic labelling (using alkaline phosphatase and peroxidase) showed that the different proteins are usually present in the same cells. A less frequent pattern of staining took the form of delicate strands of intercellular IgG.

Samples from a wide range of skin diseases were studied, but no clear relationship between positive epithelial staining and disease states was apparent. It is suggested that serum proteins may enter epithelial cells by passive diffusion, and attention is drawn to other instances (e.g. in the central nervous system and lymphoid tissue) where similar phenomena have been observed by immunoenzymatic staining techniques.

Immunoenzymatic methods for labelling paraffin embedded sections of human tissue have been increasingly used in recent years. These techniques offer the advantages that they can be used to study stored histological material retrospectively and that morphological detail can be more clearly visualized in immunoperoxidase stained sections than in immunofluorescent preparations. A wide range of cellular constituents have been studied in this way, e.g. immunoglobin, carcino-embryonic antigen and lysozyme (Taylor & Burns, 1974; Mason & Taylor, 1975; Goldenberg, Sharkey & Primus, 1976), and immunoperoxidase labelling of paraffin embedded skin biopsies has recently been reported by Shousha & Svirbely (1977).

During the course of an investigation in this laboratory into the distribution of transferrin in human tissues (Mason & Taylor, 1978), it was observed that a minority of squamous epithelial cells were strongly labelled for this protein. At the time it was suggested that these cells might possibly play an antimicrobiological role in the skin. However, more recently we have observed cells of very similar appearance staining for IgG and albumin in human squamous epithelium. This suggested that serum proteins might be non-selectively taken up by epithelial cells.

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The present investigation was undertaken to explore these observations further and to establish, by the use of double immunoenzymatic labelling techniques, whether these serum proteins are found in the same or different populations of squamous cells. Furthermore by studying samples from a range of normal and pathological tissues, we hoped to learn more of the significance of these cells. Since it is probable that immunoperoxidase techniques will be used increasingly in the future in the histological study of dermatological samples, it is of importance to clarify the cause and significance of unexpected staining reactions such as those investigated in this study.

## MATERIALS AND METHODS

### *Tissues*

Paraffin embedded blocks from fifty samples of normal and diseased skin and twelve samples of normal mucous membrane from various sites including tonsil, tongue, oesophagus, bronchus and uterine cervix, were obtained from the Surgical Histological department of the Radcliffe Infirmary. With the exception of tonsil samples, which were fixed in formol sublimate for 4–18 h, all tissues had been fixed in formol saline before processing to paraffin by routine techniques. One tonsil sample was freeze dried in a tissue freeze drier, fixed in benzoquinone vapour and paraffin embedded.

Fresh unfixed biopsies were obtained from seventeen cases covering a range of skin diseases (bullous pemphigoid, dermatitis herpetiformis, pemphigus and lupus erythematosus) and frozen for cryostat sectioning.

### *Antisera*

Rabbit antisera to human proteins (IgG, IgM, albumin, C3, lysozyme and transferrin) and swine anti-rabbit IgG were obtained from DAKO-immunoglobulins. Goat anti-human albumin was obtained from Miles Laboratories. Antialkaline phosphatase antibody was prepared in this laboratory (Mason & Sammons, 1978).

### *Antigens*

Human albumin (purity: 99%) and transferrin (purity: 94%) were obtained from Miles Laboratories. Human IgG was isolated by affinity chromatography on Sepharose protein A (Goding, 1976). Conjugation to horseradish-peroxidase by the periodate procedure (Nakane & Kawaoi, 1974) and chromatographic isolation of conjugates were performed as previously described (Mason & Sammons, 1979).

### *Immunoenzymatic labelling*

Paraffin sections were stained for IgG, albumin and transferrin using the labelled antigen technique (Mason & Sammons, 1979). In summary the method involves the successive application to dewaxed paraffin sections of unlabelled specific antiserum followed by peroxidase conjugated antigen. Antibody in the antiserum acts bivalently, linking antigen in the tissue section to the labelled antigen. The technique is inherently much more specific than any conventional immunohistological technique since no non-specific antibody in the antiserum can bind the labelled antigen and thereby lead to false positive immunoperoxidase staining. Negative controls (performed by omitting the primary antiserum) showed no staining, confirming that the conjugated antigen *per se* had no affinity for the sections.

Double immunoenzymatic staining of paraffin sections using alkaline phosphatase as the second label was performed as described by Mason & Sammons (1978). Thus for example to label simultaneously IgG and albumin the following sequence of reagents was applied: (1) rabbit anti-human IgG;

(2) swine anti-rabbit IgG; (3) rabbit anti-alkaline phosphatase plus goat anti-human albumin; (4) alkaline phosphatase plus peroxidase conjugated human albumin; (5) diaminobenzidine plus $H_2O_2$; and (6) naphthol AS phosphate plus Fast Blue BBN. Cells containing IgG were then labelled blue, while albumin containing cells were stained brown. Cells containing both proteins stain a clearly distinguishable intermediate muddy blue colour.

Cryostat sections of skin were stained using the peroxidase: antiperoxidase (PAP) technique (Mason & Taylor, 1978). The same procedure was used to stain a few of the paraffin sections for IgG, IgM, albumin and lysozyme.

## RESULTS

In the majority of sections of skin or mucous membranes positive staining for IgG and other serum proteins was observed. This staining showed two different patterns (intracellular and intercellular) which will be considered separately.

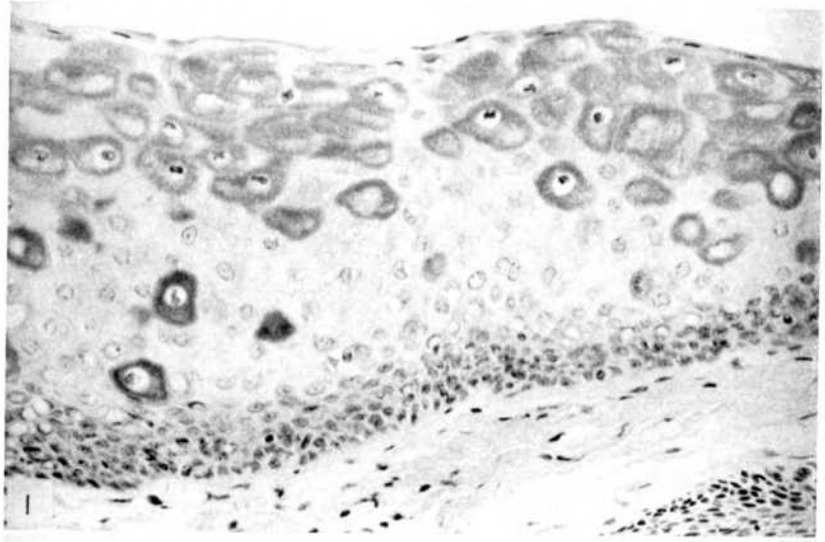
TABLE 1. Summary of staining reactions for serum proteins in different samples of squamous epithelium. The scoring for intracellular proteins (+ to +++) indicates the frequency of positive cells

| Tissue samples | No. of biopsies | Staining patterns | |
|---|---|---|---|
| | | Intracellular | Intercellular |
| Normal tissues | | | |
| Skin | | | |
| Anogenital area | 6 | +++ | + |
| Other parts of the body | 3 | + | — |
| Tonsil | 4 | +++ | — |
| Tongue | 2 | + | + |
| Oesophagus | 2 | + | + |
| Cervix | 2 | ++ | + |
| Skin diseases | | | |
| Dermatitis | 4 | + | — |
| Lichen planus | 16 | + | — |
| Lichen sclerosus et atrophicus | 2 | + | + |
| Psoriasis | 2 | + | — |
| Pemphigus | 10 | + | — |
| Bullous pemphigoid | 12 | + | — |
| Tumours | | | |
| Squamous carcinoma | 3 | +++ | + |
| Bowen's disease | 3 | + | — |
| Solar keratosis | 2 | + | + |
| Keratoacanthoma | 1 | + | + |
| Squamous papilloma | 2 | + | — |
| Seborrhoeic keratosis | 2 | + | — |
| Viral wart | 3 | + | — |
| Basal cell carcinoma | 2 | — | — |
| Intradermal naevus | 3 | +++ | — |
| Compound naevus | 4 | +++ | + |
| Malignant melanoma | 2 | + | + |
| Metaplastic epithelium (bronchus) | 2 | — | — |
| Total | 94 | | |

*Intracellular staining*

This was the most frequently encountered staining pattern, being seen in the majority of paraffin sections studied (Table 1). As seen in Figs 1–4 these cells were stained evenly throughout their cytoplasm and appeared on morphological grounds to be typical epithelial cells, except that they commonly had vacuolated pyknotic nuclei (Figs 1–4). The majority of these cells were located in the mid-zone of the prickle cell layer (Fig. 3), although in some sections positive cells could be found close to the basal layer (Fig. 4).

The frequency of positive cells in paraffin sections of normal tissues varied greatly from section to section, ranging from isolated single cells to large clusters of positive cells. In many cases only a few



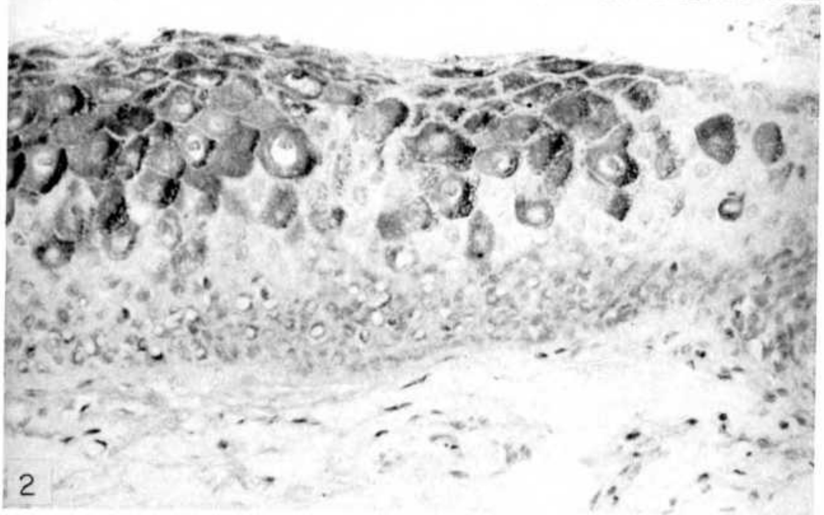


FIGURE 1. Prominent pyknotic nuclei and perinuclear vacuolation in IgG positive cells in peri-anal epithelium (× 290).

FIGURE 2. Section from same material as Fig. 1, stained for transferrin. The distribution of the positive cell population closely resembles that of IgG positive cells (× 290).

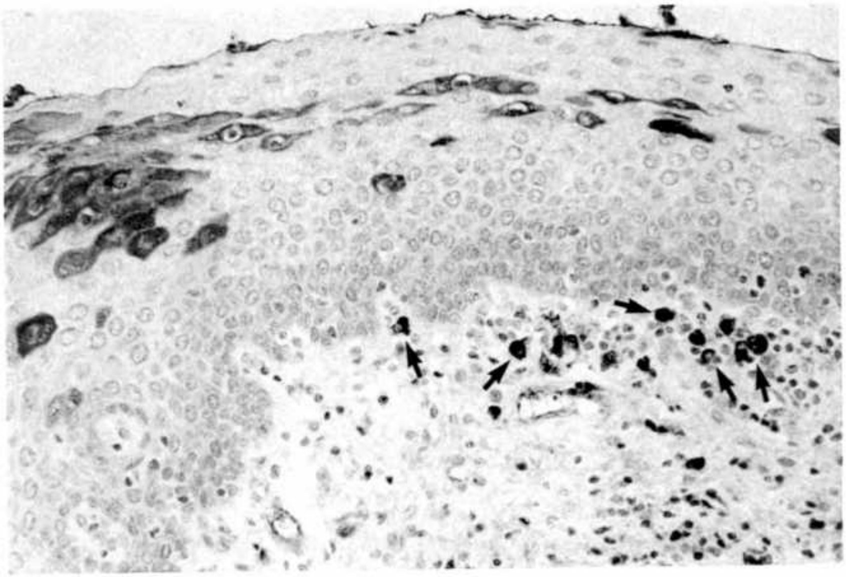


FIGURE 3. Clusters of IgG containing cells in tonsil epithelium lying principally in the mid-prickle cell layer. Note strongly positive plasma cells in tunica propria (arrowed × 290).

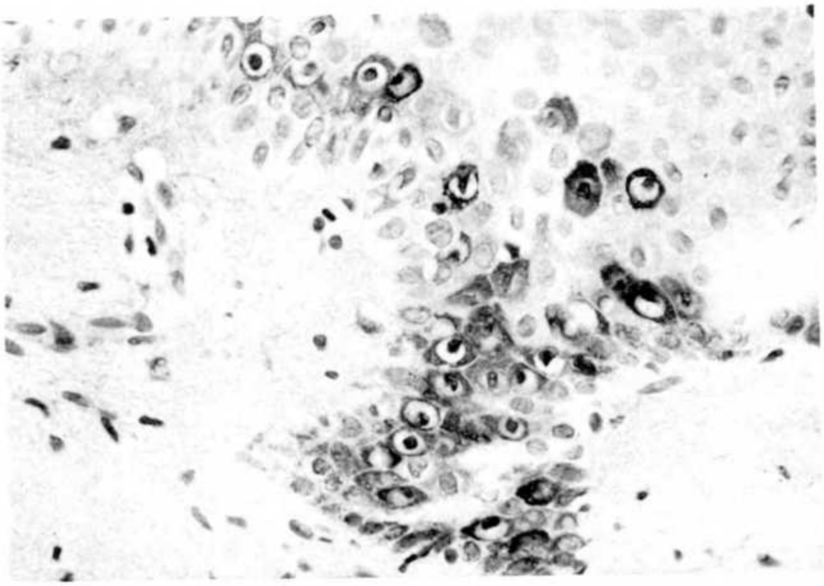


FIGURE 4. IgG containing cells in normal skin showing a suprabasal distribution (× 460).

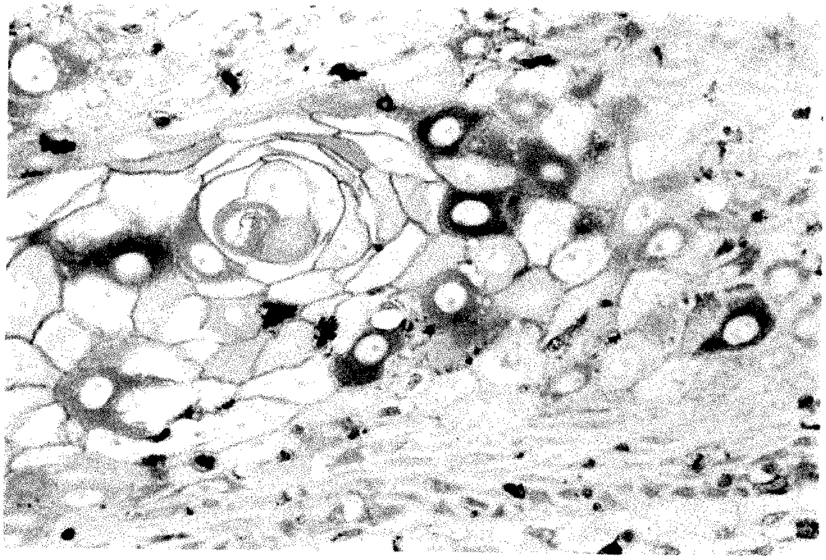


FIGURE 5. Intercellular staining for IgG in addition to intracellular positivity in squamous carcinoma of skin (×460).

positive cells could be found when the full length of a section was examined. Samples from certain sites, e.g. tonsil and anogenital skin, however, consistently contained numerous positive cells (Figs 1–3). In the former site the number of cells was highest in the vicinity of active lymphoid follicles. In the uterine cervix the positive cells were found predominantly in the portio.

When samples from a variety of benign and neoplastic skin conditions were stained for serum proteins (Table 1), there was no clear relationship between individual skin conditions and epithelial staining patterns with the exception of lichen planus samples in which colloid bodies staining for IgG, IgM and C3 were seen in addition to the intracellular staining of intact epithelial cells. Naevi, both compound and intradermal, and squamous carcinomata however were associated with the highest frequency of positive cells (Fig. 5). In addition squamous carcinomata showed intercellular staining for IgG (see below).

Intracellular staining for serum proteins was also encountered when cryostat sections from a variety of skin diseases (seventeen cases) were immunoperoxidase stained for immunoglobulins and C3. In six biopsies (three cases of pemphigus vulgaris, and single instances of dermatitis herpetiformis, lupus erythematosus and bullous pemphigoid) scattered positive epidermal cells were seen. The same staining pattern for intracellular IgG was also seen in a sample of freeze dried paraffin embedded tonsil.

Double staining for IgG and albumin was performed on paraffin sections of tonsil, anogenital skin, squamous carcinoma and naevi. In all cases the majority of cells showed unequivocal mixed staining (brownish blue) indicating the presence of both proteins within the same cells. However, a minority of cells stained blue for IgG or brown for albumin and hence contained only one of these proteins.

### *Intercellular staining*

This pattern was seen less frequently than intracellular labelling being confined to samples of oesophagus, cervix, perianal skin, compound naevi and squamous carcinomata. However, a tonsil sample which had been freeze dried before fixation and embedding showed, in contrast to conventionally processed tonsil samples, intercellular staining for IgG throughout the epidermis. Intercellular staining differed from intracellular staining (where albumin, IgG and transferrin were usually present in comparable amounts) in that only IgG was detected.

## DISCUSSION

The results reported above demonstrate that three unrelated serum proteins (albumin, IgG and transferrin) can be detected in the cytoplasm of normal and diseased human squamous epithelial cells. No previous study of this phenomenon has been reported, although Brandtzaeg, Surjan & Berdal (1978), in an immunofluorescent study of tonsillar plasma cells, commented on epithelial IgG staining very similar in appearance to the patterns illustrated above.

An important question when considering any immunohistological staining reaction concerns the specificity of the result. In conventional immunofluorescent and immunoenzymatic techniques false positive reactions (due to non-specific adherence of reagents and/or to contaminating antibodies directed against unrelated antigens) always present a potential problem. The method used in the present study (the labelled antigen technique—Mason & Sammons, 1979) minimizes the risk of unwanted staining reactions, since positive labelling occurs only when antibody of the correct specificity links peroxidase conjugated antigen to antigen in the tissue section. Antibody of any other specificity which binds to the section has no affinity for the conjugated antigen and hence cannot cause unwanted staining (Mason & Sammons, 1979).

For these reasons the specificity of the staining reactions for IgG, albumin and transferrin reported above can be accepted. It may be noted that when these proteins were detected by conventional methods (the PAP technique) identical results were obtained.

The significance of these proteins in human epithelium remains uncertain. The most probable explanation is that they represent the leakage of serum proteins into epithelial cells. Brandtzaeg *et al.* (1978) have provided direct evidence by immunofluorescence for intercellular leakage of IgG in normal human tonsillar epithelium. The association of positive cell staining with nuclear changes, such as vacuolation and pyknosis, suggests that the proteins enter degenerate cells whose surface membrane has become permeable to large molecules. Also in keeping with the leakage hypothesis is the tendency for positive cells to be most frequent in sites where the epithelium is moist (e.g. tonsil, perianal areas) and hence more likely to allow diffusion of proteins. The association of these cells with evidence of inflammation, e.g. active lymphoid centres in the tonsil and dermatitis in the skin (Mason & Taylor, 1978), provides further support for the hypothesis, since this would favour transudation of serum protein.

An argument which could be put forward against this explanation of epithelial cell staining is the fact that positive cells were surrounded by clearly negative epithelial cells (Figs 1–3), whereas on the basis of the 'leakage' hypothesis it would be expected that positive cells would be found in areas of diffuse extracellular staining representing extravasated serum proteins. However, this ignores the effect of fixation and embedding which is likely to elute and denature extracellular material. In consequence positive cells stand out strongly against a background of spuriously negative cells. The fact that intercellular IgG (in addition to intracellular IgG) could be demonstrated in freeze dried tonsil epithelium (but not in formol sublimate fixed tissue) suggests that conventional processing preferentially destroys intercellular proteins.

The findings described in this study are not unique to squamous epithelia since intracellular staining for serum proteins has been observed in immunoperoxidase studies of paraffin embedded sections of central nervous system, intestinal epithelium and lymphoid tissue (Esiri, Taylor & Mason, 1975; Bell, Piris & Mason, 1979; Mason *et al.*, 1980). The frequency of this phenomenon suggests that the results of immunoenzymatic staining of paraffin embedded material for serum proteins should be interpreted with caution and the possibility of the uptake of exogenous proteins into permeable cells always considered.

A further observation which emerged from this study was the presence of intercellular IgG (but not transferrin or albumin) in certain tissues, notably oesophagus, compound naevi and squamous carcinomata. The presence of these proteins in the latter condition might suggest the presence of host anti-tumour cell antibodies. However, the fact that an identical staining pattern was seen in non-malignant tissues (e.g. oesophagus) argues against this interpretation, and the explanation may lie in the cellular architecture in these tissues which allowed IgG to permeate between epithelial cells. Presumably the protein then binds non-specifically to cell membranes. The fact that intercellular IgG was not seen in the majority of epithelia unless special techniques to preserve it were employed (i.e. freeze drying—see above), suggests that only when relatively large quantities are present can it be demonstrated in paraffin sections of conventionally fixed tissue. We have been able to show in preliminary experiments that intercellular IgG in routine formalin fixed skin biopsies can be 'unmasked' by trypsin digestion of sections prior to staining.

In conclusion it may be said that although the results reported above may represent non-specific uptake of extracellular protein by epithelial cells the presence of a local high concentration of intracellular proteins with anti-bacterial activity, i.e. IgG and transferrin, should be taken into account when considering the immune defence capacity of the skin and mucous membranes, especially since the presence of these serum constituents was often associated with inflammatory changes in the tissue.

## ACKNOWLEDGMENTS

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# Chromosome studies in mycosis fungoides

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## SUMMARY

G-banded chromosome analysis was performed on peripheral blood lymphocytes after phytohaemagglutinin stimulation from sixteen patients with mycosis fungoides. A number of chromosome abnormalities were observed in a low proportion of blood lymphocytes in seven patients in later stages of the disease. In four patients similar marker chromosomes in multiple copies were present, some of them without a clearly visible centromere.

We conclude that a correlation exists between the presence of chromosome abnormalities in circulating blood lymphocytes and the course of the disease.

Mycosis fungoides (MF) is characterized by the presence in the skin of large abnormal lymphoid cells with indented nuclei and scanty cytoplasm. These 'mycosis cells' or 'cerebriform mononuclear cells' may invade the epidermis forming Pautrier abscesses. In later stages these lymphoid cells can also be seen in lymph nodes and visceral organs (Rappaport & Thomas, 1974; Scheffer, Meyer & van Vloten, 1979). Except for the very last stage of mycosis fungoides these lymphoid cells are not observed in the peripheral blood smear by routine examination. However, a small number of these cells can be seen in the peripheral blood using the electron microscope (Meyer *et al.*, 1977). The large lymphoid cells in the skin and lymph nodes of patients with mycosis fungoides have abnormal DNA values as was shown by cytophotometric studies (van Vloten, Schaberg & van der Ploeg, 1977; van Vloten, Scheffer & Meyer, 1979).

Cytogenetic and cytophotometric studies in Sézary's syndrome, a cutaneous T-cell lymphoma with circulating abnormal lymphoid cells, showed abnormal karyotypes with aneuploidy, polyploidy and marker chromosomes (Crossen *et al.*, 1971; Lutzner *et al.*, 1973; Bosman, van Vloten & Pearson, 1976). Cytogenetic studies in mycosis fungoides have been performed in a limited number of cases (Obara, Makino & Mikerni, 1970; Erkman-Balis & Rappaport, 1974; Fukuhara, Rowley & Variakojis, 1978; Goh, Reddy & Joisky, 1978) and also showed abnormal karyotypes with chromosome numbers ranging from 44 to 50. These studies were performed to determine the value of karyotype analysis in the interpretation of lymph node histology. The precise nature of the abnormalities remained unclear since no banding techniques were employed systematically except for the single case of Fukuhara *et al.* (1978).

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In the present study chromosome analysis was performed on phytohaemagglutinin (PHA) stimulated peripheral blood cells of sixteen patients with mycosis fungoides, using G banding. Due to too small and frequently infected tissue specimens of skin and lymph nodes in the different stages of the disease, chromosome analysis was only performed on blood lymphocytes. The results show that, although no abnormal lymphoid cells are found in routine blood smear examination, a number of chromosome abnormalities are present in a proportion of the blood lymphocytes.

## PATIENTS AND METHODS

Sixteen patients (five females and eleven males) were studied (Table 1). The diagnosis of mycosis fungoides was based on clinical and histological criteria (Lever & Schaumberg-Lever, 1975) and in most of the patients also on cytophotometric criteria (van Vloten *et al.*, 1977). The ages ranged from 42 to 77 years. The patients were staged according to Fuks, Bagshaw & Farber (1973), i.e. Stage I: mycosis fungoides of the skin; Stage II: mycosis fungoides of the skin with dermatopathic lymphadenopathy; Stage III: mycosis fungoides of the skin with lymph node involvement; Stage IV: mycosis fungoides of the skin with visceral involvement. The lymph node histology was classified as proposed by Scheffer *et al.* (1979). The follow-up period was up to 2 years. The cytogenetic studies were performed during the investigation for staging of the disease before total skin electron irradiation

TABLE 1. Clinical details and chromosome counts of analysed circulating lymphocytes in patients with mycosis fungoides

| | | | | | Chromosome counts | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Sex | Age at diagnosis | Stage | Follow-up* (months) | <43 | 43 | 44 | 45 | 46 | 47 | 48 | >48 | 92 | Total |
| Patients without chromosomal abnormalities | | | | | | | | | | | | | | |
| M.W. | F | 61 | I | 13 R | | | 1 | 4 | 25 | | | | | 30 |
| R.V. | M | 42 | I | 9 R | | | | 5 | 25 | | | | | 30 |
| L.Z. | F | 77 | II | 12 died | | 2 | 5 | 4 | 24 | | | | | 35 |
| A.W. | F | 62 | II | 17 R | 1 | | 3 | 7 | 26 | | | | | 37 |
| A.T. | M | 64 | II | 8 R | | | 2 | 2 | 26 | | | | | 30 |
| J.La. | M | 51 | II | 18 PR | 1 | | 1 | 8 | 23 | | | | | 33 |
| G.B. | M | 51 | III | 15 R | | | | 2 | 26 | | | | | 28 |
| F.V. | M | 61 | III | 15 PR | | 1 | 1 | 3 | 24 | | | | | 29 |
| H.L. | M | 60 | III | 4 PR | 1 | 1 | 2 | 2 | 23 | | | | | 29 |
| Patients with chromosomal abnormalities | | | | | | | | | | | | | | |
| J.W. | M | 55 | II | 23 R I† | 5 | 2 | 1 | 6 | 23 | | | | 1 | 38 |
| J.W. | | | | II | 1 | | 1 | 5 | 18 | | | 2 | 1 | 28 |
| A.N. | M | 72 | III | 12 died | 1 | | 2 | 6 | 25 | | | | | 34 |
| J.Lo. | M | 66 | III | 24 died | 7 | 3 | 4 | 9 | 25 | 6 | 1 | 4 | 1 | 60 |
| J.T. | F | 75 | III | 15 died I | | 1 | 1 | 4 | 24 | 1 | | | | 31 |
| J.T. | | | | II | | | 1 | 6 | 20 | 2 | | | | 30 |
| J.M. | M | 74 | III | 12 died | 1 | | 1 | 3 | 22 | | | 2 | 1 | 30 |
| J.R. | M | 69 | III | 11 PR | | | 2 | 1 | 24 | 1 | | 1 | | 29 |
| A.M. | F | 70 | IV | 2 died | 2 | 5 | | 6 | 18 | 2 | 1 | 1 | 1 | 36 |

* R = sustained remission.
PR = partial remission.
† II = repeated chromosome analysis after 7 (J.W.) and 11 (J.T.) months.

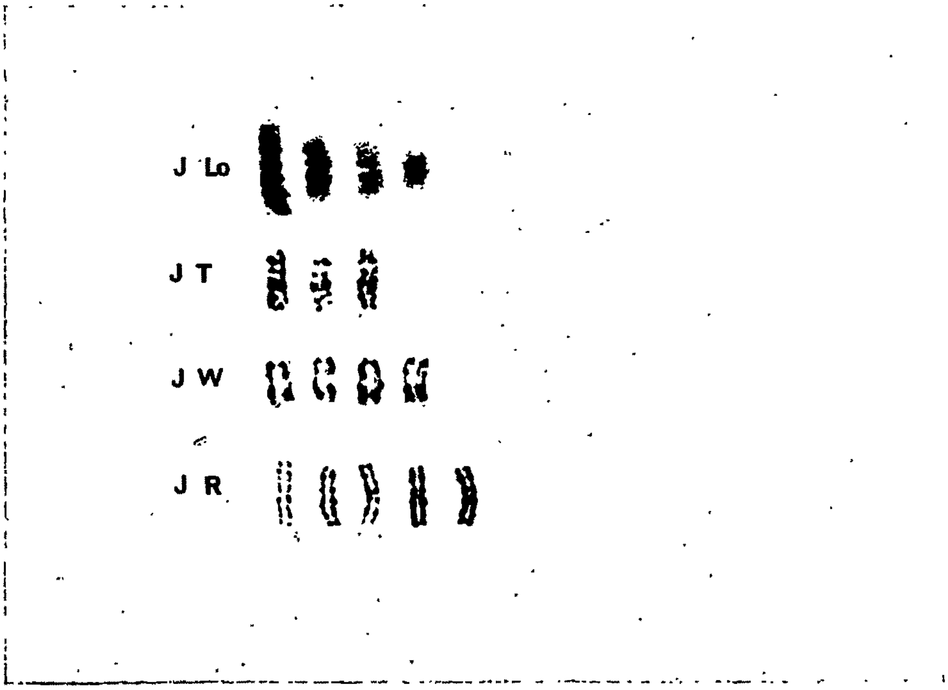

FIGURE 1. Marker chromosomes of four patients with MF.

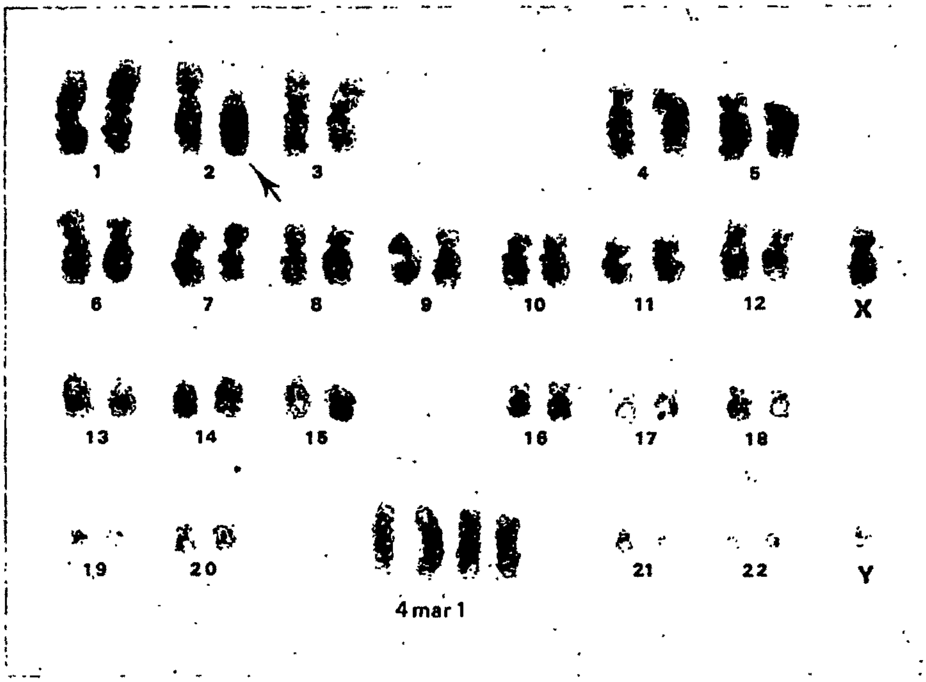

FIGURE 2. Karyogram of patient J.Lo. Arrow points to abnormal chromosome 2; in addition 4 identical marker chromosomes are present.

and/or cytostatic regimes were given. In two patients (J.T., J.W.) the chromosome analyses were repeated during the follow-up period.

For chromosome analysis 5 ml heparinized blood was obtained from each patient for a PHA stimulated blood culture technique (Bosman *et al.*, 1975). Parallel cultures without PHA stimulation were carried out in six cases. Conventional air-dried slides were G banded using two different methods (Bosman & Schaberg, 1973; Pearson & van Egmond-Cowan, 1976). The slides were examined and the chromosome number of twenty well spread metaphases counted. In addition at least ten metaphases per patient were photographed and where possible metaphases with structural and numerical abnormalities were included. Karyotyping was performed according to the recommendations of the Paris Conference (1972), supplemented with the recently described nomenclature for acquired chromosome aberrations (1979).

## RESULTS

The clinical data of the patients investigated are summarized in Table 1. Eight patients showed lymph node involvement (Stage III) and one patient (A.M.) had visceral involvement (Stage IV). After determination of the progression of the disease in each patient, treatment was started.

The results of the chromosome analysis of the PHA stimulated cultures are given in Table 1 and Figs 1 and 2. In nine patients only diploid and hypodiploid numbers were observed. None of the latter

TABLE 2. Abnormal cells (except hypodiploid cells due to random loss)

| Patient | Date of investigation | Karyotype of abnormal cells |
|---|---|---|
| J.W. | 14.03.77 | 46, XY, t (14; 18) (q13; p11) |
| | 10.10.77 | 45, XY, −13, r (3), +ace |
| | | 46, XY, −4, −9, −20, t (X; 2) (q26; p22), +3mar, 6 d min |
| | | 50, XY, +4mar1 (see Fig. 1) |
| | | 76, XYY, 2dic (16; 17) (4n−) |
| A.N. | 25.10.76 | 46, XX, t (5; 6) (p15; q15) |
| J.Lo. | 10.01.77 | 46, XX, del (10) (p11) |
| | | 50, XY, +Y, −9, +1mar1, +2mar2, +1mar3 (see Fig. 1) |
| | | 50, XY, del (2) (p11), +4mar1 (see Fig. 2) |
| | | 77, XXYY (4n−) |
| J.T. | 21.03.77 | 46, XX, 3 dmin |
| | | 47, XX, +X |
| | 14.02.78 | 46, XX, −9, +14 |
| | | 47, XX, +X |
| | | 47, XX, +14, del (20) (q11) |
| | | 48, X, −X, +3mar1 (see Fig. 1) |
| J.M. | 15.11.77 | 46, XY, −21, −22, +mar1, +mar2 |
| | | 55, XY, +X, +Y, +2, +3, −5, +10, +11, −13, +14, +17, +18, +21, +mar |
| J.R. | 07.03.78 | 52, XY, +X, +8, −21, +5mar1 (see Fig. 1) |
| A.M. | 17.01.77 | 43, XX, −4, −9, −10, −18, +mar |
| | | 47, XX, +1 |
| | | 59, XX, +2, +3, +6, +11, +12, +13, +14, +16, +19, +20, +21, +22 |

cells was recorded as abnormal since, apart from the numerical abnormalities, no rearranged chromosomes were present and no hypodiploid cells represented more than once. It cannot be concluded whether the rather high frequency of hypodiploid cells observed in these patients is due to fragility of these cells. The remaining seven patients had at least one structural or hyperploid chromosome abnormality; their karyotypes are given in Table 2. However, the majority of cells were normal in these patients also. Two patients were studied and found to be abnormal on two occasions. All unstimulated cultures revealed very few metaphases, which were very difficult to analyse, and were therefore not included in this study.

## DISCUSSION

The great majority of cells from all patients had a normal chromosome number and karyotype. This finding suggests that most T lymphocytes in patients with MF are chromosomally normal.

That the karyotypically abnormal cells are probably of T-cell origin can be concluded from the almost complete absence of dividing cells in parallel cultures without PHA stimulation. One may conclude that patients with chromosome aberrations most probably have a higher proportion of abnormal cells in their peripheral blood, the conclusion agreeing with the observation that chromosome abnormalities are predominantly found in patients in Stages III and IV ($P = 0{\cdot}01$) (Table 3).

TABLE 3. Number of patients in different stages of MF with and without chromosome abnormalities

| | Stages | | | |
|---|---|---|---|---|
| No. of patients | I | II | III | IV |
| With only normal cells | 2 | 4 | 3 | 0 |
| With at least one abnormal cell | 0 | 1 | 5 | 1 |

A rank order correlation test gives a probability value of 0·01.

During the follow-up period five of the patients in Stages III and IV died of MF, while a complete remission was achieved in only one of the remaining patients. Of the patients in Stages I and II on the other hand, only one died of MF and a complete remission was observed in five of the remaining six patients. The correlation between the presence of chromosome abnormalities and survival is evident: five out of seven patients with chromosome abnormalities died of the disease in contrast to only one out of nine patients with normal karyotypes only. Since all studies were done before cytostatic therapy was given, the observed aberrations cannot reflect adverse effects of applied potential mutagens. However, in the two patients who were also studied after electron irradiation (J.W.) and chemotherapy (J.T.) an increase in the number of abnormal cells was observed. Two general conclusions can be drawn from the observed cytogenetic changes: (1) except in patient J.T. the abnormalities do not seem to be clonal; and (2) the most consistent finding between patients is the presence of marker chromosomes in multiple copies.

In one cell of patient J.T. 3 double minutes (d min) were found, while a cell of J. W. showed 6 d min among other abnormalities. These small structures without centromere have been reported by many authors from altogether some sixty tumours (Mitelman & Levan, 1978), including two patients with MF (Erkman-Balis & Rappaport, 1974).

Multiple d min were also observed in stimulated peripheral blood of all three patients with Sézary's syndrome studied previously (Bosman *et al.*, 1976). However, some differences between the results of chromosome investigations in Sézary's syndrome and MF exist. In the former disease the (subnormal) PHA stimulation results in many karyotypically abnormal metaphases, with a wide variation between the patients. The abnormalities include a large number of structural changes resulting from translocations and a high frequency of (near) tetraploidy. In our patients tetraploid cells were only occasionally observed (Table 1).

In four of our patients with MF, multiple copies of marker chromosomes were observed in one or two cells (Fig. 1). These markers are structurally abnormal chromosomes and although they varied in size between our patients, they had in common that the centromere position could not easily be recognized. The observation of such chromosomes in patients who had d min in other cells is reminiscent of the observation of similar marker chromosomes in the SEWA mouse ascites tumour, which is known to carry many d min (Levan, Levan & Mondahl, 1978).

The absence of clonal chromosomal abnormalities in PHA stimulated blood differs from those patients hitherto studied in this respect by Erkman-Balis & Rappaport (1974), Obara *et al.* (1970) and Goh *et al.* (1978). Recent banding studies in another patient (Fukuhara *et al.*, 1978) showed only one chromosomally abnormal cell in the stimulated peripheral blood culture of a patient who otherwise had three clonal abnormalities in the lymph nodes. These latter results fit with our observation.

Different surveys of MF patients cannot be compared directly unless the clinical stage is identical and all patients are studied before therapy is given. The absence of clone formation in the peripheral blood might be explained by there being insufficient numbers of abnormal cells circulating to be detected within the sample sizes used. A second possibility cannot be excluded—that the chromosomally abnormal cells present in the circulation do not respond to PHA stimulation, which would imply that circulating MF and Sézary cells have different PHA stimulatory characteristics and would argue for two separate disease entities.

## ACKNOWLEDGMENT

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# Transient acantholytic dermatosis

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## SUMMARY

A series of twenty-four cases of transient acantholytic dermatosis (TAD) is presented. All patients were male and their eruptions tended to be more persistent than those of previously reported cases. The epidermal changes included four patterns of acantholysis, of which the pemphigus-like pattern was associated with the more persistent eruptions. Although twenty-three of the twenty-four patients had other actinic-associated skin lesions, no direct relationship between the onset of TAD and episodes of excessive sun exposure was found. Oral vitamin A treatment has been effective in some cases.

Transient acantholytic dermatosis is a primary acantholytic disorder first described by Grover in 1970. The eruption is predominantly papulo-vesicular, self-limiting, usually lasting up to several months and it occurs more commonly in men over the age of 50. The cause is not known, but eleven of the fifty-four patients reported by Chalet, Grover & Ackerman (1977) related the onset of the eruption to excessive sun exposure.

As TAD is becoming diagnosed more frequently in Western Australia, we present these cases in order to compare their clinical and histological features with those described in previous reports.

## PATIENTS AND METHODS

Transient acantholytic dermatosis was diagnosed on the basis of clinical and histological findings in twenty-four patients who presented for dermatological consultation within the 18-month period from June 1976 to December 1978.

All patients were male Caucasians, their ages at onset ranging from 32 to 86 years, the mean being 63. All had fair skin. Eleven patients stated that they always burned on sun exposure and never tanned, while thirteen said they often burned before acquiring a light tan. In twenty-three of the twenty-four patients, solar damage was evident on exposed areas ranging from several solar keratoses to severely damaged skins in which keratoses were present by the dozen, accompanied by squamous cell carcinomas and basal cell carcinomas. However, only one patient related the onset of the eruption to an episode of excessive sun exposure.

Five patients first noticed their eruptions while in hospital for myocardial infarction. Thirteen had

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hypertension as classified for their age group. Six patients had a family history of atopy and two had psoriasis. No relationship could be established between drugs and the onset or exacerbation of the eruption.

The eruption consisted of polymorphic lesions including papules, vesicles, eczematous plaques and excoriated shiny nodules. The most characteristic individual lesion, although not always present, was a shiny scalloped papule found on the trunk (Fig. 1). The eruption occurred most severely on exposed areas of the body in fifteen patients but in the other patients it was evenly distributed on exposed and non-exposed skin. All patients complained of pruritus of varied intensity and this was invariably episodic and accentuated by heat.

The duration of the eruption varied from 2 weeks to more than 10 years in one case; excluding this latter case the mean duration was 47 weeks. The patient whose eruption had lasted over 10 years had intermittent bullous phases of the disease.

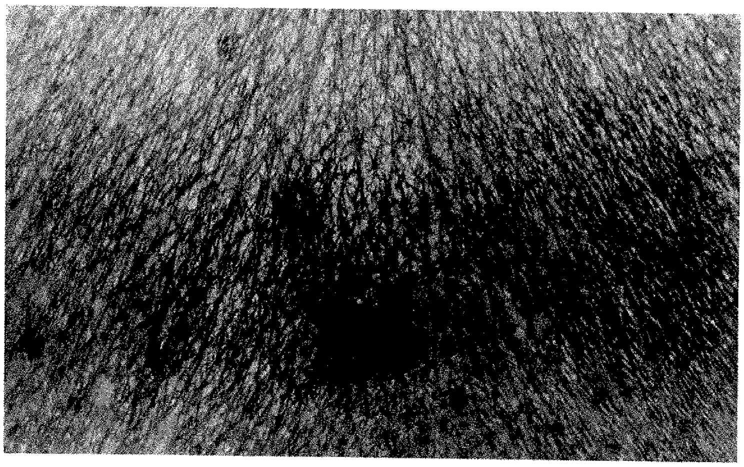

FIGURE 1. Shiny papules on trunk ( × 2·4).

*Histopathology*

Skin biopsies taken from all patients were fixed in formalin, processed by routine histological methods and stained with haematoxylin and eosin. The biopsy sites were mainly on the trunk and upper arms, well away from other lesions such as tumours.

The range of histological changes included the four main epidermal patterns described by Chalet *et al.* (1977) as resembling Darier's disease (Fig. 2), Hailey-Hailey disease (Fig. 3), pemphigus (Fig. 4), and a spongiotic type with acantholysis (Fig. 5). Common to all patterns was acantholysis, occurring in a suprabasal location in those lesions of Darier or pemphigus type, and higher in the stratum Malpighii in those of Hailey-Hailey type. A few lesions resembling pemphigus foliaceus were also seen with acantholysis involving the cells of the uppermost layers of the stratum Malpighii and the granular layer.

In the Darier pattern the acantholysis was accompanied by marked dyskeratosis, sometimes with the formation of grains and corps ronds. Milder dyskeratosis was present in some lesions of the Hailey-Hailey type and those resembling pemphigus foliaceus. Other epidermal changes seen in many cases were hyperkeratosis, acanthosis and parakeratosis, tending to be most severe in those of Darier type and least severe or absent in the lesions of pemphigus type.

In eleven cases only one epidermal pattern was present but in the remaining thirteen cases different

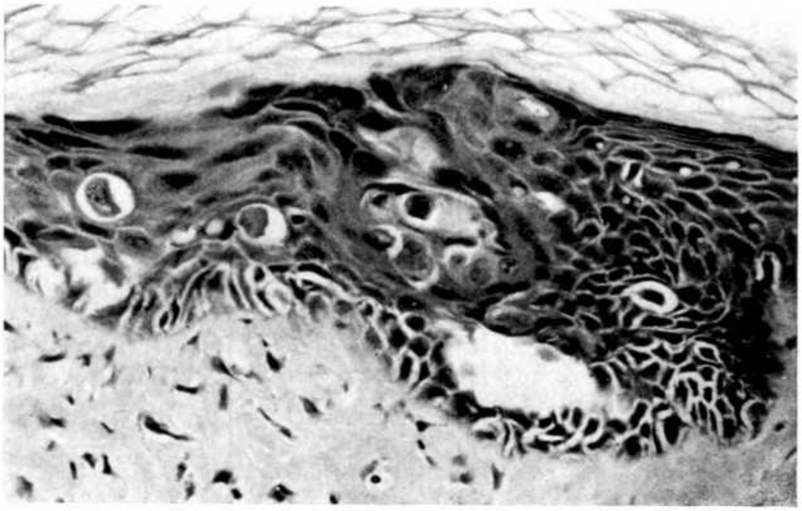

FIGURE 2. Early Darier-like lesion (× 320).

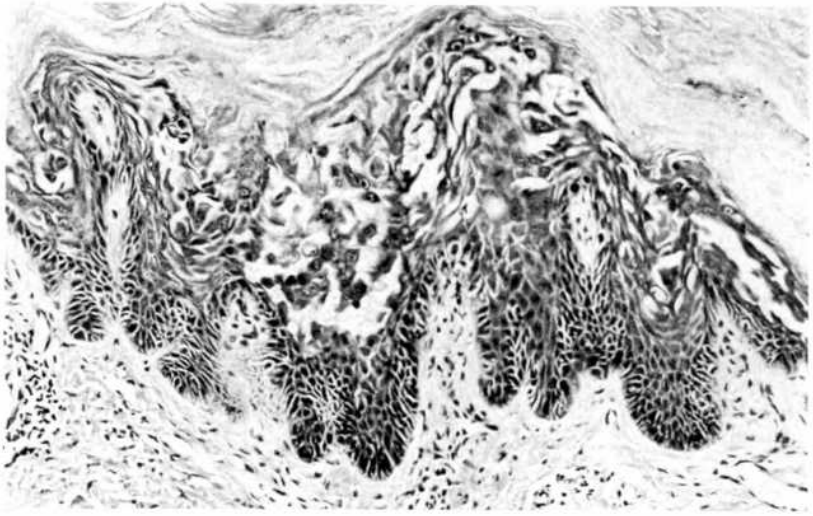

FIGURE 3. Acantholysis involving most of the stratum Malpighii, resembling Hailey-Hailey disease (× 80).

patterns occurred in separate foci in the same biopsy specimen. The Darier pattern was seen alone in four cases, the pemphigus type in four, and spongiosis with acantholysis in three. The Hailey-Hailey type was seen only in combination with other patterns. The Darier's disease pattern was the most common overall, occurring in combination with others in ten cases, five of which were of pemphigus type. In one biopsy all four patterns were present, occurring in separate foci.

A superficial, mainly perivascular dermal infiltrate of lymphocytes and histiocytes accompanied all the epidermal changes with the exception of some cases of pemphigus type which were of bland appearance. In thirteen cases, eosinophils were also present in the dermal infiltrate but only in small numbers and they were not related specifically to any of the patterns of epidermal change. Solar elastosis was present in seven biopsies, in association with all four epidermal patterns.

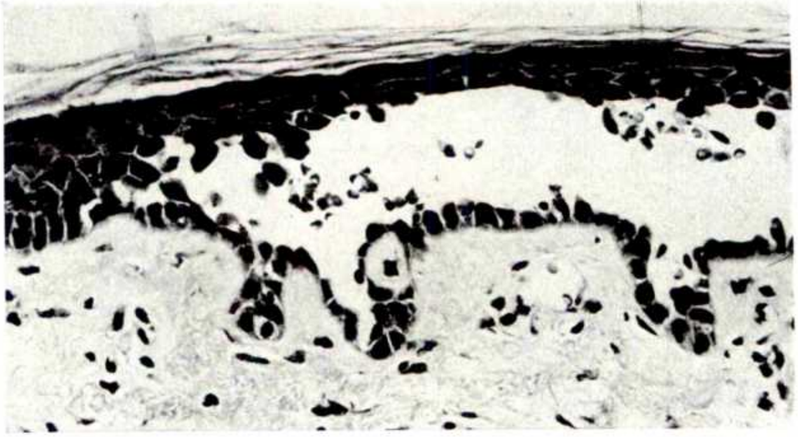

FIGURE 4. Pemphigus-like lesion (×320).

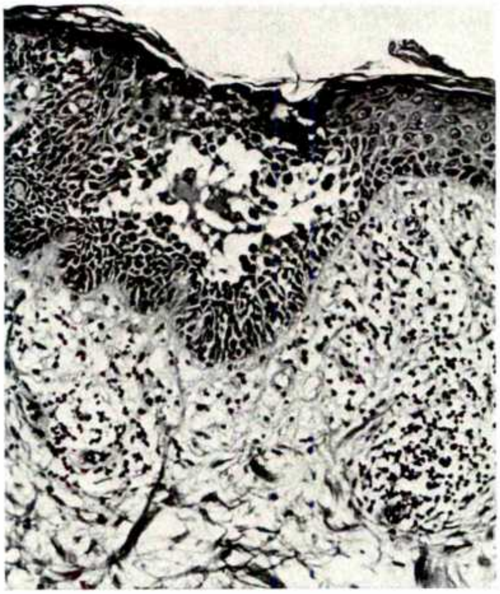

FIGURE 5. Spongiosis with acantholysis (×80).

*Immunofluorescence*

Direct and indirect immunofluorescence tests were consistently negative in the ten patients in whom they were performed.

*Treatment*

In the mildest cases simple antipruritics served as satisfactory treatment. In most cases, however, topical fluorinated steroids were necessary to control the pruritus and in two patients intermittent courses of oral steroids were necessary. Dapsone was tried without success in six patients and antihistamines were of limited value in controlling the pruritus.

## DISCUSSION

The salient clinical features of transient acantholytic dermatosis in these cases emphasize its association with chronic solar damage to exposed skin, the persistence of the eruption in some cases compared with the transient nature of those initially reported by Grover (1970), and its predilection for males.

Although twenty-three of twenty-four patients had other epidermal lesions for which chronic exposure to strong sunlight has strong associations, for example solar keratosis, basal cell carcinoma, and squamous cell carcinoma, only one patient related the onset of the eruption to a distinct episode of excessive sun exposure. This might be explained, at least partially, by the generally sunny climate in this state and by the outdoor lifestyle of the inhabitants. The annual average daily hours of bright sunshine in Western Australia ranges from 7·3 in the south to 9·7 in the northernmost areas, the average in Perth where the vast majority of the population lives being 7·5 h/day. Habitual exposure to strong sunlight, as indicated by the large numbers of skin tumours in these patients, would probably render them less likely to remark on periods of exposure which might seem excessive in other countries. However, many of our patients presented in winter so that our findings do not support a direct relationship with acute episodes of sunlight exposure.

The association of exacerbations of pruritus with hot conditions is more in keeping with the finding of Heaphy, Tucker & Winkelmann (1976) that four of their seven patients with benign papular acantholytic dermatosis related exacerbation of their eruption to activities that induced sweating.

The persistence of some cases is in accord with the re-evaluation of transient acantholytic dermatosis by Chalet *et al.* (1977). In their view the cases of persistent acantholytic dermatosis reported by Simon, Bloom & Ackerman (1976) and those of benign papular acantholytic dermatosis by Heaphy *et al.* (1976) remain within the category of transient acantholytic dermatosis, allowing for exceptional examples of longer than usual duration. Our findings tend to support that opinion.

The total male predominance in these twenty-four patients compares with a 31:9 male:female ratio in the series reported by Chalet *et al.* (1977). There seems to be no obvious reason for this different sex distribution but as transient acantholytic dermatosis becomes recognized more frequently, more detailed epidemiological analysis should lead to clarification of this and other aetiological aspects of the disorder.

The histological characteristics of transient acantholytic dermatosis that have been recognized to date are probably not pathognomonic but they are sufficiently suggestive to permit a firm diagnosis to be made when considered in conjunction with the distinctive clinical presentations. The combination of different patterns of epidermal change occurring in the same biopsy or in separate biopsies from the same patient, especially when the changes occur in very small, circumscribed foci, appears to offer the best histological evidence for the diagnosis. However, several patterns of acantholysis may occur in association with other acantholytic processes such as pemphigus vulgaris (Chalet *et al.*, 1977), so that the final diagnosis must depend always on both clinical and histological findings, with immunofluorescence studies to exclude pemphigus.

When seen in isolation, the histological features of each pattern of acantholysis are not specific for transient acantholytic dermatosis. In particular, the well-developed Darier-like pattern is indistinguishable from that of true Darier's disease, as has been emphasized by Grover & Duffy (1975). The light microscopic recognition of acantholysis within spongiotic vesicles is thought to be a helpful clue to the diagnosis of transient acantholytic dermatosis (Chalet *et al.*, 1977), or even unique to this disease (Ackerman, 1978).

The pemphigus-like pattern seen in our cases has usually shown only a very narrow, slit-like suprabasal cleft containing a few acantholytic cells. None of the clefts contained eosinophils and when eosinophils were present in the dermis they were sparse. This bland appearance helped to distinguish these changes from pemphigus vulgaris, the distinction being confirmed by negative immunofluorescence tests. We have seen this pattern of acantholysis in the skin close to tumours and other disorders including one case of cutaneous secondary syphilis, and this apparently incidental finding has been more common in our experience than the Darier-like change described by Ackerman (1972) as focal acantholytic dyskeratosis. The patients whose biopsies showed the pemphigus pattern tended to have the most persistent eruptions, including that which has lasted for 10 years, the mean duration of the other three cases being 82 weeks.

The Hailey-Hailey-like pattern was seen only in combination with other changes and occurring only in tiny foci.

The range of acantholytic changes, with and without dyskeratosis, observed in these twenty-four cases include patterns which would, in our view, be indistinguishable from those described by Heaphy *et al.* (1976) as benign papular acantholytic dermatosis and the two cases of Gisslen & Mobacken (1978), described as acute adult-onset Darier-like dermatosis.

Treatment with oral vitamin A (150,000 units three times daily for 2 weeks or longer) in ten patients, including five from this series and five later cases, whose biopsies included all four main epidermal patterns, has produced a satisfactory response, as recently reported by Rohr & Quirk (1979). Other forms of treatment of transient acantholytic dermatosis have been unsatisfactory with the exception of oral corticosteroid therapy. It was decided to use vitamin A in these patients because of the histological similarities between some cases of transient acantholytic dermatosis and Darier's disease and the good response of some patients with Darier's disease to vitamin A.

It is our impression that TAD is more common than has been generally accepted by dermatologists, and that the diagnosis is now being differentiated from others such as acne, miliaria, folliculitis and prurigo simplex through the wider recognition of its clinical and histological features.

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# South American pemphigus foliaceus: electron microscopy and immunoelectron localization of bound immunoglobulin in the skin and oral mucosa

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## SUMMARY

Five skin and two oral biopsies from patients with South American pemphigus foliaceus (SAPF) were studied by electron and immunoelectron microscopy for the ultrastructural localization of bound immunoglobulin in epidermal and oral lesions.

Electron microscopy showed the tonofilament-desmosome complex to be preserved in the various layers of the epidermis. Immunoglobulin was bound over the plasma membrane and permeated the desmosomal junctions both in the skin and oral mucosa, thus suggesting that pemphigus antibodies are attached to the glycocalyx. It appears that the initial injury in SAPF acantholysis involves the glycocalyx and that it might be caused by interaction with intercellular antibodies present in the patient's serum.

South American pemphigus foliaceus (SAPF), or 'fogo selvagem' (wild fire), is a disease endemic in Brazil, but also reported in Northern Argentina, Paraguay, Venezuela and Peru, and which differs from Cazenave pemphigus foliaceus in its course and epidemiology (Brown, 1954; Proença & Castro, 1971).

The purpose of this paper is to report a combined transmission electron microscopy study and immunoelectron localization of bound immunoglobulin in the skin and oral mucosa in SAPF in an attempt to understand the pathogenesis of the lesions.

## PATIENTS AND METHODS

Five patients with generalized SAPF were examined. The average age was 31·8 years. Three of them

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were males, the youngest being 20 and the oldest 55 years old. Two of them were females in their twenties. Skin biopsies were performed in all these patients and oral biopsies in two during the active stage of their eruption. An attempt was made to select lesions of recent origin. Four patients had been without any therapy at all. One had recently abandoned therapy after 4 years of treatment and had a clinical relapse of the disease.

In all patients, circulating antibodies to intercellular substance were demonstrated by immunofluorescence testing on guinea-pig oesophagus (Beutner *et al.*, 1968).

### *Electron microscopy*

Half of the biopsy was cut into small pieces of 2–3 $mm^3$, submitted to routine double fixation with 1% glutaraldehyde and osmic acid, embedded in Araldite and stained with uranyl acetate and lead citrate.

### *Immunoelectron microscopy*

The other half of the biopsy was immersed in PLP (periodate-lysine-paraformaldehyde) fixative, and horse-radish peroxidase (HRPO)–labelled Fab′ of anti-human IgG was used for the demonstration of bound IgG, according to McLean & Nakane (1974).

### *Light microscopy*

From all patients skin biopsies were also taken from lesions in different stages, and routine 5 μm paraffin sections stained by H & E were prepared.

### *Controls*

Two biopsies of normal skin and one oral biopsy from the cheek mucosa were taken from three patients in their thirties, undergoing either orthopaedic surgery or tumour resection, and used as controls for light, electron and immunoelectron microscopy.

## RESULTS

### *Light microscopy*

Histological findings were as previously described by Alayon (1948) and Furtado (1959).

### *Electron microscopy*

In all cases the dermis appeared unchanged and the basement membrane intact. The basal cells with the aid of the anchoring filaments adhered to the basement membrane in a normal fashion. The desmosomes were present in the basal cell region and Malpighian area.

In the Malpighian region with bulla formation, isolated or rows of acantholytic cells were observed either with partial (Fig. 1a and b) or complete loss of desmosomes (Fig. 1a). Acantholysis was initiated by the separation of the non-specific junctions. Subsequently desmosomal junctions become disrupted. In degenerated cells tonofibrils were discernible as bundles without proper orientation. Dyskeratosis of cells of the Malpighian layer was represented by tonofilaments arranged as an irregular network of interwoven bundles. Keratohyalin granules were present in many of these dyskeratotic areas. Lamellar bodies were present in the Malpighian and granular layers, similar to those seen in normal skin. The mitochondria were occasionally swollen, but complete epidermal cell degeneration was not observed.

Oral mucous membrane, examined in two cases, did not differ from the normal on morphological grounds.

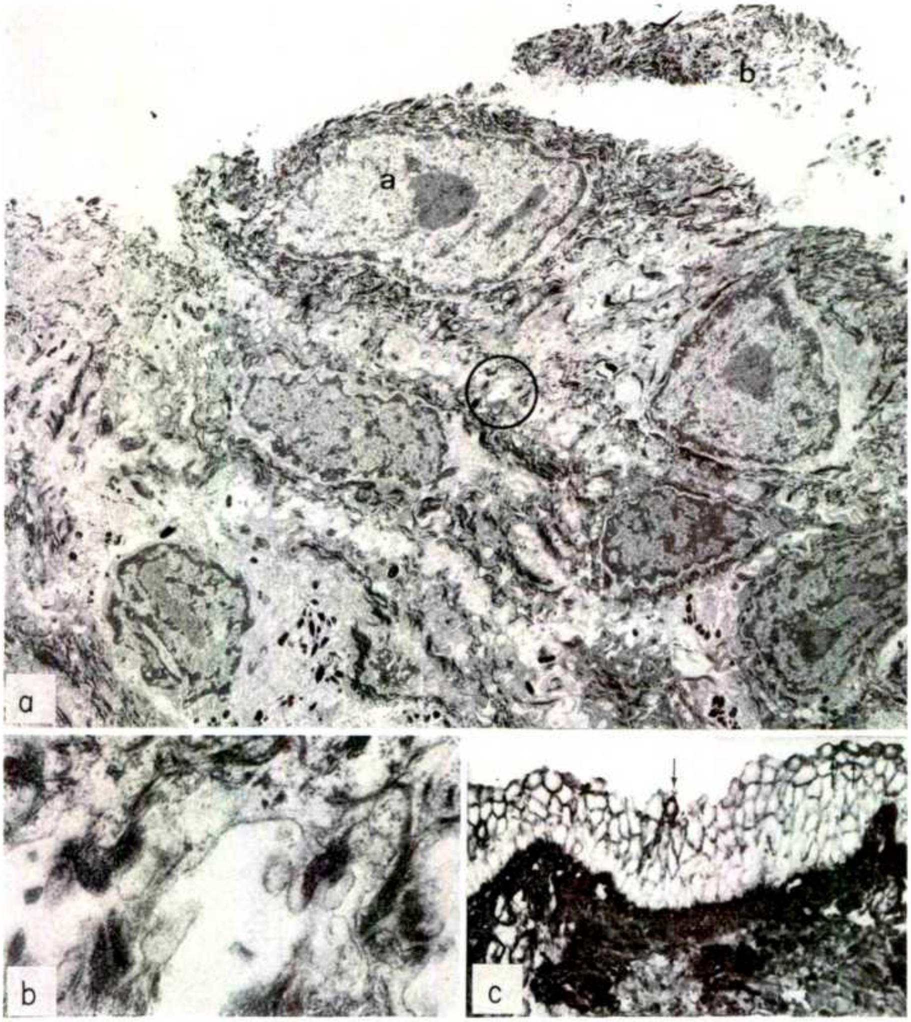

FIGURE 1. (a) Epidermis of a patient with SAPF. Floor of a bulla located high up in the epidermis. Cell a is partially detached from the neighbouring cells. Cell b, completely free in the lumen, is represented by part of its cytoplasm (× 4000). Circle shows low power of Fig. 1b. (b) Area delimited by the circle in micrograph 1a showing preserved tonofilament–desmosome complex at the base of cell a (× 40,000). (c) Light microscopy showing immunological reaction product surrounding epidermal cells of the various layers. The floor of a bulla can be seen with acantholytic cells encircled by heavy deposits of the reaction product (arrows) (× 250).

### *Immuno-light and electron microscopy findings*

Light microscopy of the skin and oral mucous membrane marked for demonstration of bound IgG showed the marker deposits fixed to the intercellular substance (Figs 1c, 2a and d). Where early acantholysis was present heavy dark deposits were seen surrounding the injured cells (Fig. 1c). Otherwise the deposits appeared uniform around cells of the various layers.

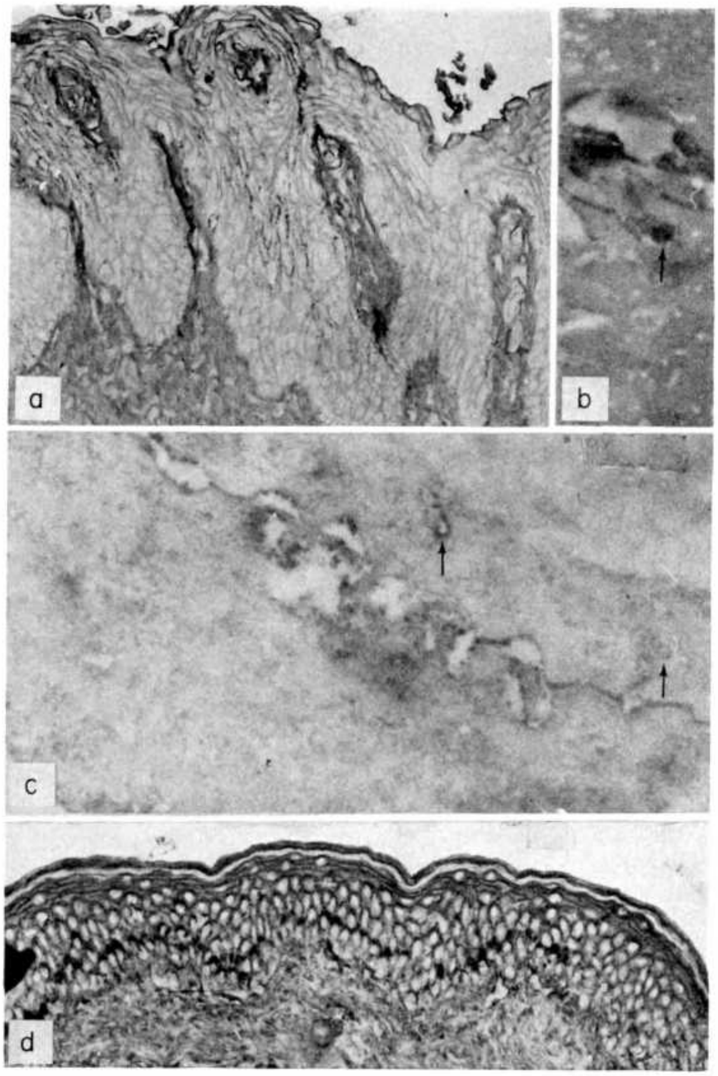

FIGURE 2. (a) SAPF. Oral mucosa. Light microscopy showing immunological reaction product encircling cells of the various epithelial layers (× 250). (b) Immunoelectron microscopy of oral epithelium showing oval dense structures (arrow), apparently in the intercellular space (× 30,000). (c) Immunoelectron microscopy of the oral epithelium showing oval structures delimited by the reaction product (arrows) in the peripheral cytoplasm of two adjacent cells. Reaction product is seen over the cytomembranes, occupying the spaces between epithelial cells (× 30,000). (d) SAPF. Light microscopy of the immunological reaction in a paralesional area of the epidermis showing uniform cell delimitation of the various layers by the reaction product (× 250).

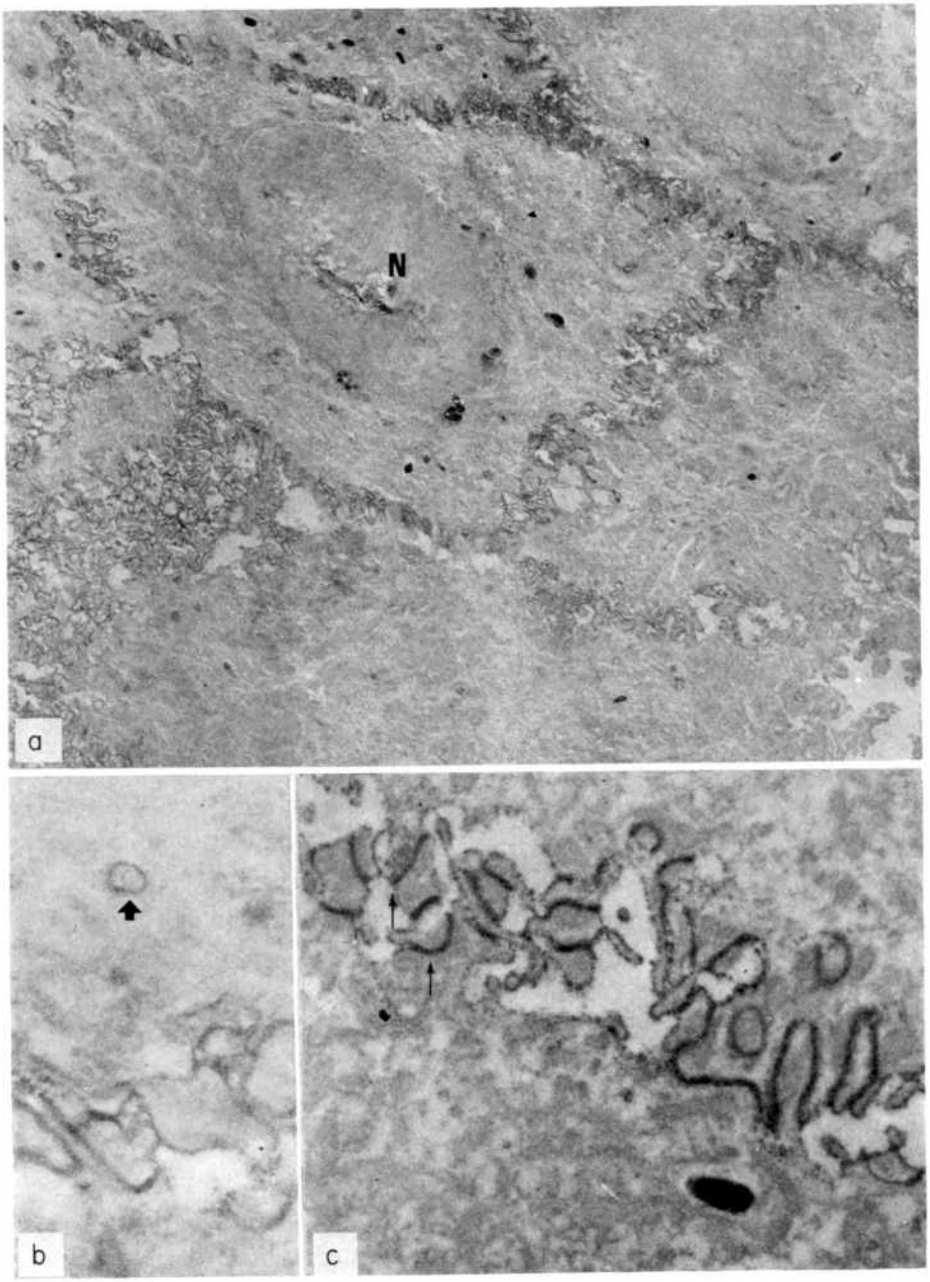

FIGURE 3. (a) SAPF. Immunoelectron microscopy of epidermal cells. Reaction product is seen occupying intercellular spaces between keratinocytes. N designates cell nucleus ( × 10,000). (b) Oval structure delimited by the reaction product in the cytoplasm of epidermal cells (arrow). Reaction product is seen over the cytomembranes, covering non-specific junctions ( × 25,000). (c) Reaction product over the cytomembranes and permeating desmosomes (arrows). Oval structures in the cytoplasm are transversely cut interdigitations between adjacent cells ( × 30,000).

Immunoelectron microscopy disclosed the electron dense immunological reaction product restricted to the epidermis and oral epithelium, coating the cytoplasmatic membrane of the cells (Fig. 3a). Desmosomes were permeated by the reaction product without any preferential accumulation within them (Fig. 3c). In areas of the epidermis where the cells had separated the reaction product formed irregular deposits along the cytomembranes. In the Malpighian layer and oral mucosa occasional cells exhibited, near the cytomembranes, round or oval small structures coated by the reaction product (Fig. 3b). In oral mucosa similar structures were discernible also in the intercellular space, adhering to the cytomembrane (Fig. 2b and c).

Controls, as viewed both by light and immunoelectron microscopy, were devoid of reaction product.

## DISCUSSION

Wilgram, Caulfield & Madigic (1964) pointed to the changes of the tonofilament–desmosome complex as the earliest epidermal cell alteration in Cazenave pemphigus foliaceus.

In the only previous report on the electron microscopy of SAPF (Barros, 1972) the primary event was also described as an alteration of the tonofilament–desmosome complex.

Our findings, however, disclosed that throughout the epidermis, including the main site of the lesions, desmosomes were present. Oral mucosa showed a normal pattern.

Konrad, Stingl & Holubar (1977), studying a single case of SAPF, disclosed IgG deposits in the intercellular spaces in the epidermis. We confirmed these findings and showed, even in the absence of local clinical manifestations, deposits in the intercellular spaces of the oral epithelium. The reaction product permeated the desmosomes without preference for these structures. Such findings suggest, as previously pointed out in pemphigus vulgaris (Wolff & Schreiner, 1971; Honigsmann *et al.*, 1975), that in SAPF antibodies are attached to the glycocalyx, which is involved in cell-to-cell interaction. Since, with the exception of acantholytic cells, no marked injury to the tonofilament–desmosome complex was detected, it appears that the initial site of the lesion in SAPF acantholysis is the glycocalyx and, further, that it might be the result of an interaction with an intercellular antibody present in the patient's serum.

Probably in SAPF there occurs what has already been hypothesized in pemphigus vulgaris, i.e. antigenic substances, produced through the action of a virus or other unknown pathogenic agent, are discharged into intercellular spaces between potentially keratinizing epithelia. Alternatively the antigenic substances, instead of being produced locally, could originate somewhere else, having a preferential affinity to the intercellular space material of the epithelia (Beutner *et al.*, 1965; Hashimoto & Lever, 1967, 1970).

Reaction products are seen marking round or oval structures at the peripheral cytoplasm of cells of the keratinizing epithelia and oral mucosa. Such corpuscles, chiefly when they are located deeply in the peripheral cytoplasm, can be interpreted as lamellar bodies. Their structure did not suggest viral particles. In the keratinizing epithelia, however, when they are close to the cell membrane, the possibility of them being transverse cuts of cell interdigitations cannot be discarded. If such structures are lamellar bodies this suggests that they might be antigenic and also responsible for the formation of auto-antibodies.

In SAPF it is puzzling that though the location of the intraepidermal bullae is high up in the epidermis no preferential intercellular deposits were observed in that region. This contrasts with the findings of Bystryn, Abel & De Feo (1974) who used immunofluorescence techniques in four of ten patients with Cazenave pemphigus foliaceus, and found autoantibodies specifically reacting with antigens present in the subcorneal intercellular substance of the epidermis.

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# Mixed connective tissue disease characterized by speckled epidermal nuclear IgG deposition in normal skin

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## SUMMARY

Four African female patients are described, who presented with the features of systemic sclerosis. Overlapping features of lupus erythematosus or dermatomyositis were present in three cases but were not prominent. Direct immunofluorescence of uninvolved skin revealed a particulate (or speckled) epidermal nuclear staining, with specificity for IgG. In view of the reported association between this finding and mixed connective tissue disease, these patients were treated with corticosteroids and marked improvement occurred in all cases. The usefulness of this investigation in making the distinction between systemic sclerosis and mixed connective tissue disease and in indicating a potentially effective form of therapy is discussed.

Mixed connective tissue disease is a clinical entity recently defined by Sharp *et al.* (1971). Patients with this condition show overlapping features of systemic lupus erythematosus (S.L.E.), systemic sclerosis and dermatomyositis. This syndrome is usually benign, and is characterized by an excellent response to corticosteroid therapy. It is the serological features that indicate this condition to be a distinct clinical entity (Sharp *et al.*, 1972). Typically, the anti-nuclear factor is found in high titre, and is deposited in a speckled or particulate manner. It was found that this antibody was directed against a saline-soluble or extractable nuclear antigen (E.N.A.). However, many patients with the typical features of S.L.E. possess similar antibodies. It was then found possible to differentiate the antibodies in the two conditions by treatment with ribonuclease. In mixed connective tissue disease, the E.N.A. antibody was greatly suppressed, whereas it was unaffected in systemic lupus erythematosus. It was therefore assumed that in mixed connective tissue disease the antigen is a ribonucleoprotein, whilst in S.L.E. the antigen appears to be a glycoprotein, thought to be identical with the Sm antigen.

Direct immunofluorescence studies of outwardly normal skin are valuable in the investigation of patients with S.L.E. (Burnham & Fine, 1971). In such studies, particulate or speckled nuclear staining has been reported in various rheumatic diseases. This epidermal nuclear staining was found to corre-

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late with high titres of antibodies to E.N.A.—to both the ribonucleoprotein and the glycoprotein varieties (Gilliam & Prystowsky, 1977; Prystowsky & Tuffanelli, 1978).

We describe four African patients, presenting as systemic sclerosis with certain unusual features. These cases were all found to demonstrate particulate epidermal staining for IgG. In view of the association between this staining pattern and mixed connective tissue disease, these patients were treated with corticosteroids, and all showed a marked clinical improvement.

## PATIENTS AND METHODS

Four young African female patients were studied, each presenting with an initial diagnosis of systemic sclerosis. All four cases had sclerodermatous skin changes and swollen hands with sclerodactyly, together with Raynaud's phenomenon, arthralgia and lymphadenopathy. Three complained of dysphagia. Unusual features suggestive of an overlap syndrome were muscle weakness and wasting in two patients, and a transient erythematous rash over the malar regions in another.

All patients were subjected to skin biopsies, samples being taken from the apparently uninvolved skin of a light-exposed area (dorsal forearm) and a light-protected area (buttock). Frozen sections were stained using standard immunofluorescence techniques (Beutner *et al.*, 1973), utilizing commercially prepared fluorescein-labelled anti-sera to IgG, IgA, IgM, fibrin and C1q, C3 and C4 components of complement.

### *Case* 1

A 35-year-old African woman presented in June 1978 with an 18-month history of progressive arthralgia and limitation of movement in the hands, elbows, knee and ankle joints, together with Raynaud's phenomenon. Two weeks prior to presentation, she had developed gangrene of her left index finger. She also complained of muscle weakness and dysphagia. On clinical examination, there was skin thickening and induration involving all her limbs with areas of hyper- and hypopigmentation. She was unable to open her mouth widely, and there was patchy alopecia. Contraction deformities occurred in the elbows, knees and many finger joints, and gangrene was present at the extremity of the left index finger. Moderate muscle wasting was observed. Lymphadenopathy was generalized.

Laboratory studies showed an E.S.R. of 120 mm/h, hyper-gammaglobulinaemia, creatinine phosphokinase 208 iu/l (normal 15–90), aldolase 15 iu/l (normal 1·4–3·1), positive anti-nuclear factor (speckled pattern, titre 1:256), normal barium swallow but abnormal oesophageal motility studies, a muscle biopsy showing degeneration of muscle fibre and a mononuclear cellular infiltrate, and skin biopsies showing a granular band of IgM at the dermo-epidermal junction and speckled nuclear staining for IgG over the epidermal cells (Fig. 1).

She was treated with prednisone 60 mg daily, tapering down to 10 mg daily. After 6 months, mobility of the fingers and other joints had markedly increased, noticeable softening of the skin had occurred and muscle weakness had improved with muscle enzyme levels back to normal.

### *Case* 2

A 28-year-old female was first seen in August 1976, with joint stiffness, Raynaud's phenomenon, lymphadenopathy, tightening and thickening of the skin of the hands and an erythematous rash over the malar regions of the face. Over the next 18 months the fingers became atrophied, bound down and sclerosed, and the process spread to the forearms and upper chest. Laboratory investigations demonstrated an E.S.R. of 40 mm/h, positive rheumatoid factor (1:40) and anti-nuclear factor (1:256), creatinine clearance of 86 ml/min, renal biopsy showing diffuse non-proliferative mesangial sclerosis

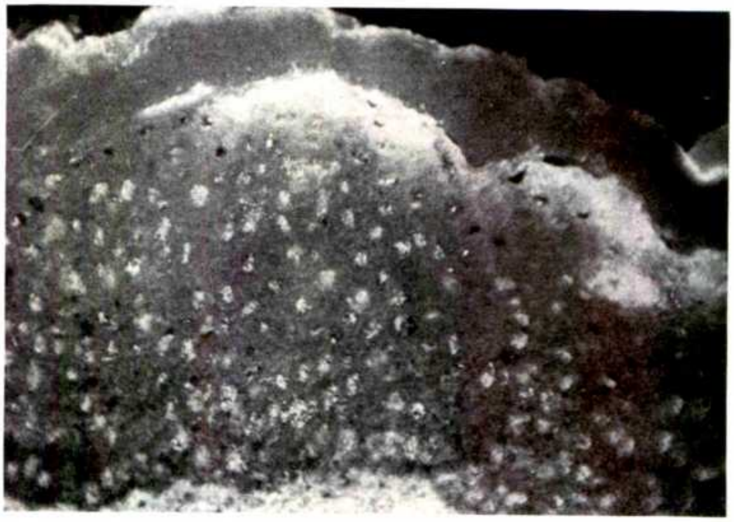

FIGURE 1. Case 1. Speckled nuclear staining for IgG over the epidermal cells (× 200).

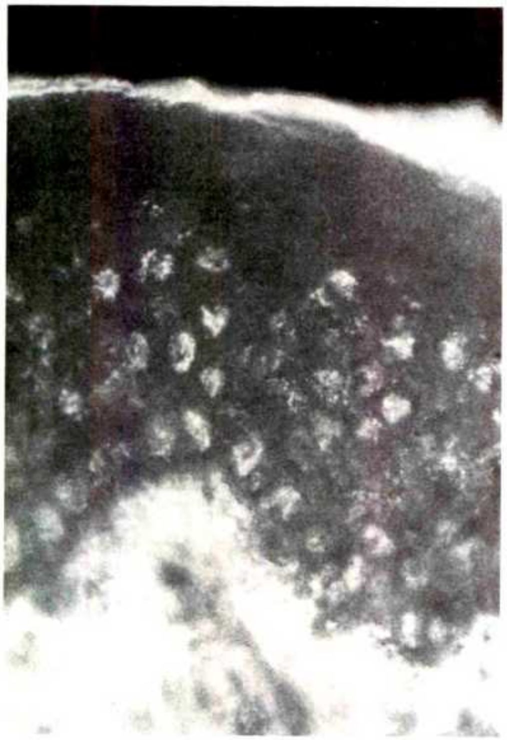

FIGURE 2. Case 2. Speckled epidermal nuclear staining for IgG. The polyvalent anti-serum used here also demonstrates a 'lupus band' at the epidermal junction, staining for IgM (× 320).

and skin biopsies showing an IgM band at the dermo-epidermal junction and speckled epidermal nuclear IgG staining (Fig. 2).

Prednisone 30 mg daily produced an immediate clearing of the facial rash, and within a month there was virtual resolution of the sclerosed skin and markedly improved joint function. The E.S.R. had dropped to 8 mm/h, the rheumatoid and anti-nuclear factors were undetectable, but a skin biopsy taken at this stage still demonstrated speckled nuclear staining.

### *Case* 3

A 38-year-old female presented in October 1978 with a 6-month history of swelling of the hands and feet, and Raynaud's phenomenon. She also complained of weight loss and muscle weakness. Her fingers were swollen, tightly bound and sausage-shaped with a cyanotic hue and necrotic and ulcerated areas. More extensive gangrene was present over the toes. The mouth was tight and furrowed. There was extensive lymphadenopathy with tenderness. Laboratory investigation showed Hb 11·8 g/dl, E.S.R. 38 mm/h, positive anti-nuclear factor and rheumatoid factor (1:80), hypergammaglobulinaemia and raised IgG, positive cryoglobulins, marginally raised creatinine phosphokinase and aldolase, acro-osteolysis of the toes, delayed oesophageal emptying, and speckled nuclear IgG fluorescence of the skin. She was treated with prednisone 40 mg daily, and after 4 months there is no muscle weakness or weight loss, but the sclerosis of the skin has improved only moderately.

### *Case* 4

A 35-year-old woman presented in November 1978 with a 3-month history of arthralgia, muscle weakness, difficulty in swallowing and loss of weight. She had Raynaud's phenomenon, swollen fingers with indurated bound-down skin. sclerodermatous change over the lower face and mouth, patchy alopecia and pigmentary change. There was wasting and weakness of the proximal musculature. Hb was 12·6 g/dl, E.S.R. 70 mm/h and there was hypergammaglobulinaemia, a positive antinuclear factor (speckled pattern), raised muscle enzymes (C.P.K. 268 iu/l) and muscle biopsy showing degenerative changes with focal inflammation. There was delayed emptying on barium swallow and marked oesophageal atonia. Speckled nuclear IgG fluorescence was seen in the epidermis. Prednisone was commenced at a dosage of 60 mg daily. Within 2 months, muscle function had greatly improved with normal muscle enzyme levels and ESR of 15 mm/h. The dysphagia had resolved, and the skin was markedly less sclerodermatous. The anti-nuclear factor remained positive, however, and the speckled nuclear IgG fluorescence was again demonstrated in the epidermis.

## DISCUSSION

Systemic sclerosis is a condition notoriously refractory to all forms of treatment. Although corticosteroids have been advocated, there is little evidence to suggest that they have any beneficial function.

The four cases described were all initially diagnosed as systemic sclerosis, and corticosteroid therapy was not at first considered. However, three of these cases developed features, which although found in systemic sclerosis, are more commonly seen in other collagen disorders. The epidermal nuclear speckled immunofluorescence, the 'lupus band' and the anti-nuclear factor, speckled in high titre, were considered to be suggestive of an overlap syndrome. For these reasons, therapeutic trials of corticosteroids were initiated with gratifying results. Until recently, it has been possible to detect antibodies to E.N.A. in only a few world centres. Gilliam & Prystowsky (1977), demonstrated the good correlation between the ribonuclease sensitive E.N.A. antibody, particulate nuclear staining and mixed connective tissue disease.

In systemic sclerosis, positive immunofluorescence findings are not frequently seen in the skin. Our four cases with particulate nuclear staining demonstrated by their natural history and response to corticosteroid therapy that they belong to a distinct variant of the condition, probably identical with Sharp's mixed connective tissue disease.

The purpose of this paper is to illustrate the importance of investigating patients with systemic sclerosis in order to exclude a diagnosis of mixed connective tissue disease. The presence of antibodies to ribonuclease-sensitive E.N.A. must be regarded as the definitive test in establishing this diagnosis, but especially where this investigation is not available, direct immunofluorescence of the skin provides an easily performed alternative. A counter-current electrophoresis method for E.N.A. has recently been set up here, and we have been able to do this on two of the cases. Case 2 was positive (1:64) before treatment and negative during remission. Case 4, after partial remission, was positive (1:32).

The satisfactory response of our patients to corticosteroid therapy corresponds to the experience of Sharp and other workers. Steroid treatment may not have been considered if the original diagnosis of systemic sclerosis had been allowed to stand. We therefore believe that it is essential to investigate all cases of systemic sclerosis by serological or immunofluorescence techniques, in order to distinguish cases of mixed connective tissue disease that may prove to be amenable to corticosteroid therapy.

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# Histochemical staining of sunburn cells for sulphhydryl and disulphide groups: a time course study

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## SUMMARY

Ultraviolet light (UV)-exposed rabbit skin was stained with a fluorescent thiol reagent or haematoxylin and eosin to investigate a time relationship between histochemical stainings for sulphhydryl (-SH) and disulphide (S-S) groups and sunburn cell (SBC) production. SBCs appeared by 8 h after irradiation, increased and showed a peak at 48 h, then declined to zero by 96 h. SBCs showing -SH staining increased to a maximum of 41–59% at 24–48 h and declined to 26–45% by 72 h, whereas S-S-stained SBCs were only a few at 8–24 h and then increased up to 65–85% by 72 h. The results seem to indicate that S-S linkage may occur in the process of SBC production.

Sunburn cells (SBCs) are eosinophilic staining cells seen in the epidermis of ultraviolet light (UV)-exposed skin (Rook & Wilkinson, 1972). As reported by Woodcock & Magnus (1976) in mouse skin, SBCs appear in epidermis by 8 h after irradiation with UV-B and are then totally eliminated by 60–72 h. Numerous biochemical or enzymological responses are known also to occur in epidermis following UV irradiation (Johnson & Daniels, 1969). They include changes in amounts of sulphhydryl (-SH) and disulphide (S-S) groups (Kusuhara & Knox, 1962; Ogura & Knox, 1964). Daniels, Brophy & Lobitz (1961) showed that SBCs stained more deeply than adjacent epidermal cells for both -SH and S-S groups. However, the time course of the UV-induced histochemical changes was not examined.

In this study, we have undertaken semi-quantitative assessments of -SH and S-S stains in SBCs at several time intervals after UV exposure, using a new fluorescent histochemical reagent, N-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM). DACM has no fluorescence by itself but strong and specific blue–green fluorescence (exciting wave length (λex) 400 nm; emission wave length (λem) 485 nm) appears only after binding to -SH groups of molecules (Kanaoka *et al.*, 1970, 1973). Specificity of the histochemical staining of tissue-bound sulphhydryl groups with this reagent has been completely studied (Ogawa *et al.*, 1979).

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## MATERIALS AND METHODS

The back of five albino rabbits was depilated and 3 days later a part of the skin was exposed to three 20 W fluorescent sunlamp tubes (Toshiba FL 20SE) for 4 (approximately a minimal erythema dose), 8 and 12 min at a distance of 10 cm. The light source emits rays mainly between 280 and 320 nm (UV-B), peaking at 300 nm. Punch biopsies of the skin (5 mm in diameter) were undertaken at 8, 24, 48, 72 and 96 h after exposure. A depilated and non-exposed area of back skin was also biopsied at the same times.

Tissues were cut and half was fixed with 10% formalin for haematoxylin and eosin (H & E) stain. The other half of the tissues was quick-frozen and 6 μm cryostat sections were stained with (1) H & E, and (2) DACM according to the method of Ogawa *et al.* (1979). Briefly, protein-bound -SH groups were stained with DACM after free -SH groups were washed out. To stain S-S groups, -SH groups first were blocked by N-ethylmaleimide (Sigma). Then, S-S linkages were opened by dithiothreitol (Sigma) and stained with DACM.

Formalin-fixed sections were used for observing histopathological changes occurring in UV-exposed skin. Approximate SBC counts were obtained by enumerating the number of SBCs through epidermis of three different H & E-stained frozen sections. The number of SBCs showing -SH or S-S staining was counted using three DACM-stained sections under a Nikon Fluophot fluorescence microscope, equipped with epi-illumination with a 200 W super high pressure mercury lamp, an excitor filter (IF 385–425) which excites wavelengths between 385 and 425 nm, and barrier filters which suppress waves below 450 nm. After the observations with the fluorescence microscope, the slides were mounted off and restained with H & E to confirm that fluorescence for -SH or S-S groups was localized in SBCs.

## RESULTS

The time course of SBC counts is illustrated in Fig. 1. SBCs appeared in living layers of the epidermis by 8 h. SBC counts rapidly increased during 8–48 h to show a peak at 48 h. The epidermis was partly damaged and crusted by 72 h in 4 and 8 min exposed animals and even by 48 h in the 12 min exposed

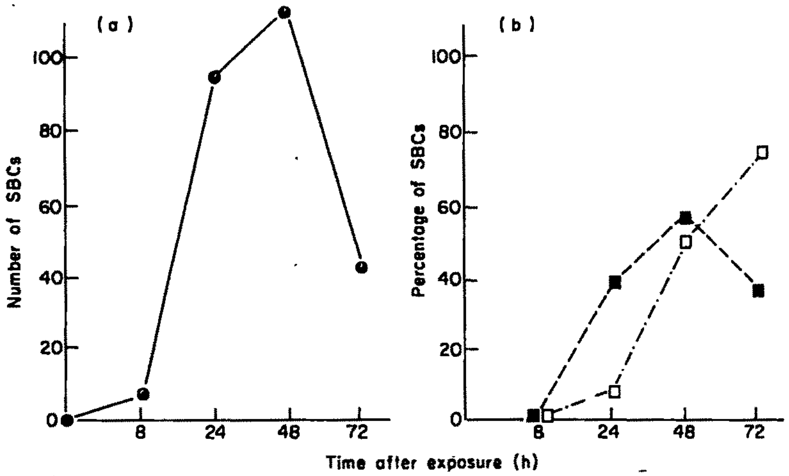

FIGURE 1. The time relationship between SBC production and SBC counts showing -SH or S-S staining after 8 min UV-exposure. In 4 min and 12 min UV-exposed groups, similar time courses were also obtained. (a) (●—●) SBC counts; (b) (■- - -■) per cent SBCs with —SH groups; (□- · -□) per cent SBCs with S—S groups.

group. SBCs thus were difficult to identify in later stages, although they seemed to occur in upper living and horny layers after 48 h. A few SBCs were also seen in the dermis at 48 and 72 h with infiltrates of mononuclear leukocytes and eosinophils. SBC counts seemed to decline by 72 h. Skin specimens taken at 96 h after exposure showed apparently normal epidermal structures without any SBC. No histopathological changes were observed in specimens taken from depilated and non-exposed skin throughout the experimental period.

In sections of non-exposed skin stained with DACM, fluorescence for -SH groups was seen mainly in the cytoplasm of living layers, while fluorescence for S-S linkages was demonstrated only in the intercellular spaces of the horny layer (Fig. 2). In UV-exposed skin, globular and homogeneous fluoresecence for both -SH and S-S stainings was observed in SBCs (Fig. 3). No other significant differences in intensity and pattern of -SH and S-S stainings were noticed between UV-exposed and non-exposed skin specimens.

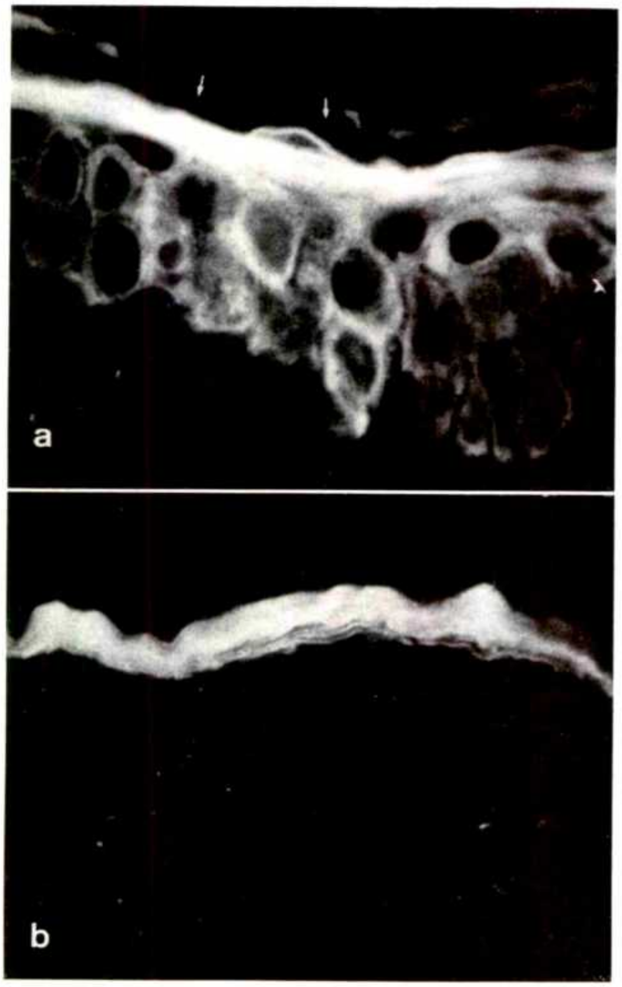

FIGURE 2. (a) —SH staining in non-exposed rabbit skin. Fluoresecence is observed in the cytoplasm of living layers and is minimal in the horny layer (arrows) (× 720). (b) S—S staining in non-exposed rabbit skin. Linear fluorescence is seen only in the horny layer (× 720).

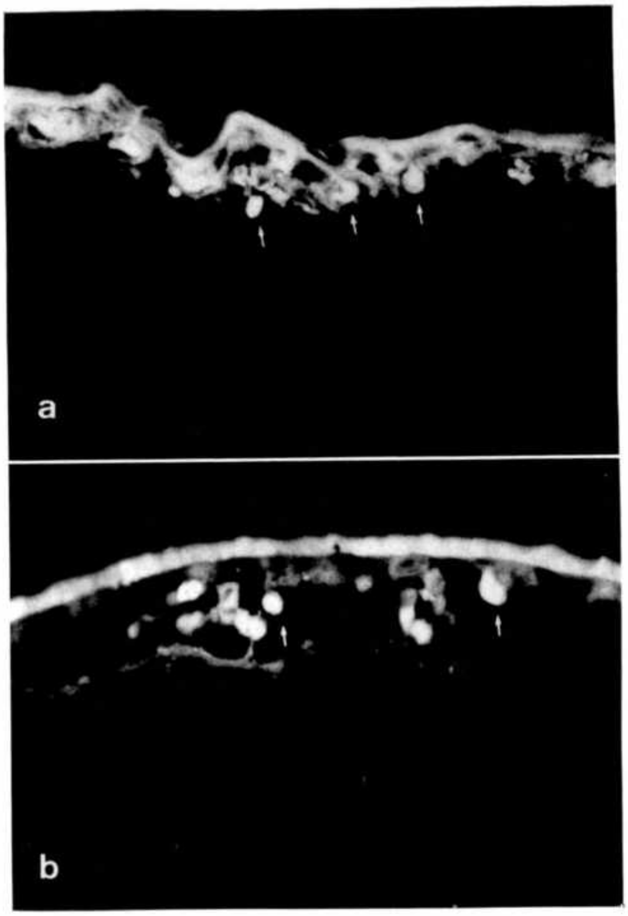

FIGURE 3. (a) —SH staining in 8 min UV-exposed rabbit skin at 24 h after irradiation. Note globular and brilliant fluorescence in SBCs (arrows) (× 360). (b) S—S staining in 8 min UV-exposed rabbit skin at 48 h, showing fluorescence in the horny layer and epidermal SBCs (arrows) (× 360).

In the time course studies, the number of SBCs showing -SH staining increased after 8 h, became 41–59% of the total at 24–48 h and then declined to 26–45% at 72 h. S-S-stained SBCs, in contrast, were less than 10% of the total between 8 and 24 h, gradually increased and attained a maximum of 65–85% at 72 h.

## DISCUSSION

In non-exposed rabbit skin, -SH groups were localized mainly in living layers of the epidermis, whereas S-S linkages were seen only in the horny layer, as seen in human skin (Ogawa *et al.*, 1979). Sulphhydryl cross-linking seemingly takes place at the junction between living and horny layers. In UV-exposed rabbit skin, however, we found S-S bonds also in the epidermal living layers, localized in SBCs, indicating that S-S linkages may occur here after UV exposure.

Recently Ogawa & Taneda (1979), using DACM staining, showed S-S bonds in individually keratinized cells but not in normal keratinocytes in squamous cell carcinoma. SBCs show by electron microscopy a tonofilamentous ultrastructure which may represent UV-induced individual cell keratinization (Wilgram *et al.*, 1970; Olson, Gaylor & Everett, 1974). Although the significance of S-S staining in SBCs is difficult to establish, it is possible that oxidization of -SH groups occurs in abnormal keratinization processes.

Utilizing the semiquantitative counting method, we found that the number of SBCs showing -SH staining was higher than those with S-S bonds during the first 48 h after exposure, while the relationship between -SH and S-S-bearing SBCs was reversed at 72 h. These UV-induced histochemical changes in SBCs seem to correlate with the biochemical alteration in whole epidermal -SH and S-S concentrations reported by Kusuhara & Knox (1962) and Ogura & Knox (1964), who used microamperometric titration in human or rat skin after a single UV exposure. In our study, no apparent difference in intensity of -SH and S-S stainings was found in epidermis except in the SBCs. It therefore seems that the histochemical changes in SBCs may partly account for transient alteration in -SH and S-S concentrations in UV-exposed skin.

## ACKNOWLEDGMENTS

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# Clinical value of serum $\gamma$-glutamyl transferase estimation in porphyria cutanea tarda

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## SUMMARY

Serum $\gamma$-glutamyl transferase was elevated fourfold in all thirty-four new cases of porphyria cutanea tarda. Phlebotomy treatment decreased it. The twelve patients in relapse showed a considerable increase, while those in remission tended to a gradual elevation in the enzyme over the course of years. $\gamma$-Glutamyl transferase is a useful auxiliary parameter in porphyria cutanea tarda particularly in untreated cases.

Some years ago we reported (Adjarov & Ivanov, 1973) that serum $\gamma$-glutamyl transferase ($\gamma$-GT, EC 2.3.2.2) was increased in 90% of patients with porphyria cutanea tarda (PCT), exceeding both in frequency and degree the changes in the other routinely estimated liver enzymes. In the present communication we report our observations on how this investigation varies during the course of the disease and with treatment.

## MATERIALS AND METHODS

Thirty-four males with PCT, aged 23–63 years (mean 46), have been investigated. At first they all had vesiculo-bullous eruptions, skin fragility and changes in pigmentation on exposed parts of the body. The main aetiological factor in all of them was prolonged alcohol consumption. In addition, fourteen of them had had occupational contact with lead, eight had had viral hepatitis and two had been treated with tuberculostatic drugs in the past. All patients were treated by phlebotomy as described by Ippen (1961) with an average 3·4 ± 1 l (mean ± s.d.) of blood withdrawn. Later twelve of them had relapses—increased excretion of uroporphyrin in the urine was noted in all, and two patients also had skin manifestations. The remaining twenty-two cases, after treatment by phlebotomy, were in a state of remission lasting from 5 months to 5 years.

Porphyrin metabolism was evaluated by the estimation of uroporphyrin (Gutniak & Dancewicz, 1958) and coproporphyrin (Rimington, 1961) in the urine, and total serum porphyrins (Schlenker, Davis & Kitchel, 1961).

$\gamma$-GT was determined by the method of Kulhánek & Dimov (1966). The data on $\gamma$-GT in PCT

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patients before treatment were compared to those in eighty males with chronic alcoholism, but who revealed no clinical signs of porphyria. Their mean age was 44 years (30–68). Thirty-one had abnormal needle or laparoscopic liver biopsies (cirrhosis, eleven; chronic hepatitis, twenty) and forty-nine had a normal liver parenchyma or merely steatosis.

## RESULTS

The treatment by phlebotomy led to pronounced clinical improvement. The parameters of porphyrin metabolism decreased ($P<0{\cdot}01$): urinary uroporphyrin from 1610±153 nmol/24 h (mean±s.e. mean) to 306±28 nmol/24h (normal range 0–36); urinary coproporphyrin from 533±79 nmol/24 h to 176±26 nmol/24h (normal range 15–150); and total serum porphyrins from 68±6 μg/l to 23±4 μg/l (normal range 0–8).

All untreated patients with PCT had elevated serum γ-GT. The mean value (83±10 iu) was over four times higher than the upper limit of normal (20 iu). There is considerable similarity between the values in PCT patients and in the alcoholics with liver involvement (88±14 iu). The alcoholics without any hepatic lesion had appreciably lesser changes (28±3 iu).

After venesection therapy, γ-GT activity decreased. Only one out of thirty-four patients showed an

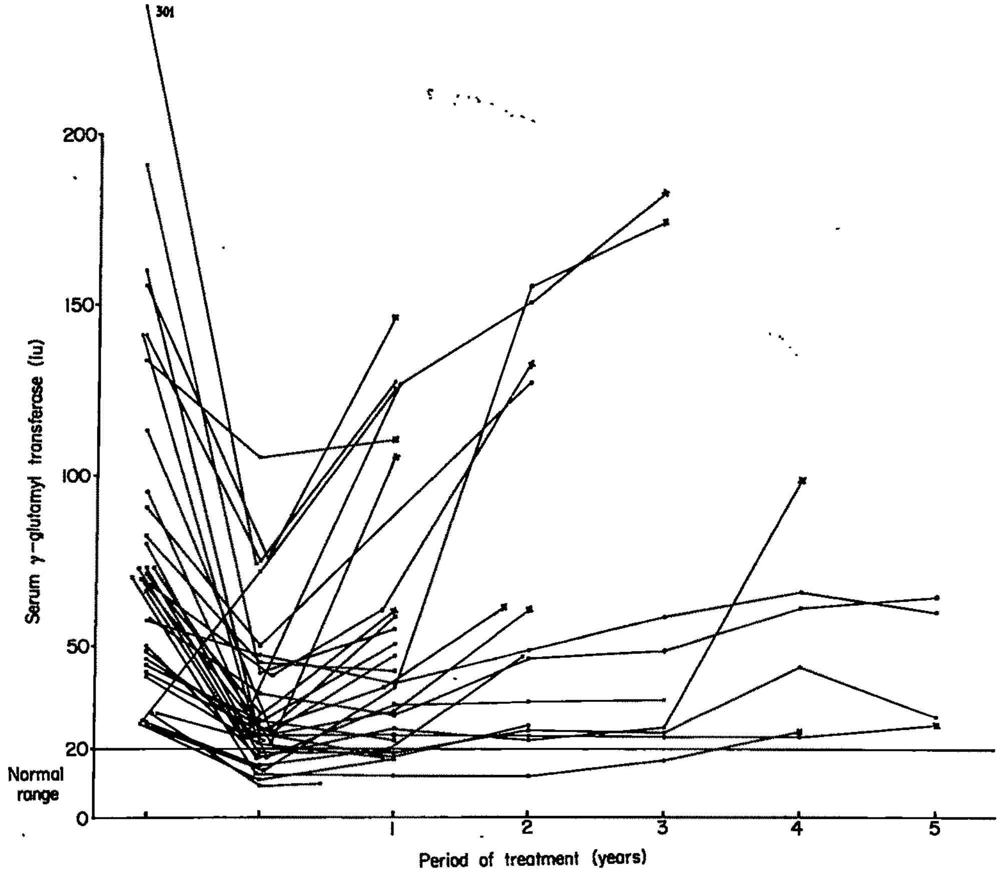

FIGURE 1. Values of serum γ-GT in thirty-four patients with PCT, treated by phlebotomy. (×) Results established during relapse.

increase. Most cases in remission had some increase of γ-GT but this was more apparent (96 ± 15 iu) when the patient relapsed (Fig. 1).

## DISCUSSION

All patients in the active stage of PCT displayed elevated γ-GT activity in the serum as has been established by others (Topi & Gandolfo, 1976; Simon *et al.*, 1977; Tarnow, 1978). In the other hepatic porphyrias we have rarely found an increased serum γ-GT.

The rise of γ-GT in PCT is probably conditioned by two main factors—the induction of enzyme activity in the liver under the influence of ethanol on the microsomes (Rosalki & Rau, 1972) and the increased permeability of the hepatocyte membrane resulting from the liver damage (Adjarov & Ivanov, 1973; Wu, Slavin & Levi, 1976; Simon *et al.*, 1977). The role of the latter is apparent from the obvious resemblance between γ-GT values in PCT patients and in chronic alcoholics with pronounced hepatic involvement. The decrease of enzyme activity at the end of the therapy is probably due to the abstention from alcohol (Lamy *et al.*, 1975) as well to the improvement in liver function (Ippen, 1977). The elevation during relapses is probably connected with renewed alcohol consumption, which was certainly the case in eight out of twelve patients. It is an open question as to what determines the gradual increase in γ-GT in the cases during lasting remission.

Following the serum γ-GT during different stages of PCT shows its clinical significance in untreated cases. Its importance in treated patients is less, due to the tendency to increase both in relapses and in prolonged remissions. The determination of serum γ-GT in PCT is a useful adjunct to the estimation of uroporphyrin and the other parameters of porphyrin metabolism.

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## *Pharmacology and Treatment*

# Dyes and preservatives in oral antihistamines

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## SUMMARY

Antihistamines are commonly used in the treatment of urticaria. Some antihistamines contain dyes and preservatives which have themselves been shown to produce or exacerbate urticaria; the presence of these substances is not indicated in the usual formularies and a list of commonly prescribed antihistamines is presented, detailing the azo dyes, other dyes and preservatives contained therein.

Asthma (Chafee & Settipane, 1967), angio-oedema (Samter & Beers, 1968), vascular purpura (Criep, 1971), and chronic urticaria (Juhlin, Michaëlsson & Zetterström, 1972; Michaëlsson & Juhlin, 1973; Thune & Granholt, 1975) may be provoked or aggravated by ingested substances contained in food, drugs or beverages.

At present, the commonest implicated substances are the azo-dyes (Chafee & Settipane, 1967; Ros, Juhlin & Michaelsson, 1976; Thune & Granholt, 1975), benzoates (Michaëlsson & Juhlin, 1973) and salicylates (Juhlin *et al.*, 1972). The pyrazole aniline dye tartrazine (BPC 1954) (CI Food Yellow 4; Colour index No. 19140; FD Yellow No. 5) is used widely in the food, drug and cosmetic industries and has been extensively investigated and shown to be associated with these untoward phenomena. Tartrazine is used predominantly as a yellow colouring agent but may be found as a component of several colours including lemon, lime, blue, brown and green. The other dyes known commonly to produce adverse reactions are New Coccine and Sunset Yellow (Michaëlsson & Juhlin, 1973). At present insufficient evidence has been accumulated directly to implicate other dyes in this group. Although commonly added during processing azo-dyes are not found naturally in foodstuffs; salicylates occur naturally in significant amounts in a wide variety of foods (Leist & Banwell, 1974) but the amount of naturally occurring benzoates is low in comparison with the amounts added as preservatives.

The mechanism of these reactions is uncertain as is the threshold amount required to evoke a response. It has been suggested that trace amounts only may suffice and Juhlin *et al.* (1972) have demonstrated reactions after the ingestion of as little as 0·1 mg of tartrazine.

In chronic urticaria allergy to foods was detected in some 2% of patients attending hospital (Champion *et al.*, 1969). Exacerbations in response to oral provocative testing with azo-dyes and benzoates have been demonstrated in up to 50% of chronic urticaria patients (Ros *et al.*, 1976). With tartrazine,

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TABLE 1. Common names of 'coal tar' colouring agents

| | |
|---|---|
| Approved for foodstuffs in EEC: | |
| *Ponceau 4R | |
| *Carmoisine | |
| *Amaranth | (FD & C Red No. 2) |
| Erythrosine BS | (FD & C Red No. 3) |
| *Tartrazine | (FD & C Yellow No. 5) |
| *Sunset Yellow FCF | (FD & C Yellow No. 6) |
| Quinoline Yellow | |
| Green S | |
| Indigo Carmine | (FD & C Blue No. 2) |
| Patent Blue V | |
| * Black PN | |
| Not approved for foodstuffs in EEC: | |
| *Orange G | |

*Azo dye.

positive reactions have occurred in 13–30% of patients tested (Michaëlsson & Juhlin, 1973; Warin & Smith, 1976). Aspirin has been found to produce a reaction in 70% of patients sensitive to azo-dyes or preservatives (Ros *et al.*, 1976). In addition, small numbers of patients with asthma have been shown to react to benzoates (Juhlin *et al.*, 1972) and in 8–15% of cases to tartrazine (Settipane & Paduapakkam, 1975). Azo-dyes and benzoates have been shown to cause a vasculitic purpura (Criep, 1971) and azo-dyes worsen contact dermatitis in paraphenylenediamine hypersensitive subjects (Baer & Leider, 1949).

In the U.K., EEC regulations governing colouring matters in food were adopted in 1973. Among the colouring agents permitted are a variety of synthetic 'coal tar' dyes. These synthetic dyes which, by virtue of their stability, have largely replaced their natural counterparts, can be broadly divided into acid and basic dyes. The acid dyes form salts with the negative ion as the coloured component. The majority of the permitted dyes are sodium salts of sulphonic acids and a great number of these are azo-dyes which by definition contain the azo chromophore $-N=N-$.

The permitted 'coal tar' dyes are listed in Table I and can be seen to include tartrazine, an azo salt of sulphonic acid. With the exception of FD and C Red No. 2 the dyes indicated under the FD and C Code are those permitted for use in food within the U.S.A. It appears to be general practice in the pharmaceutical industry to restrict colours used in drugs to those approved for food and it is probable that this policy will become mandatory in the future.

Antihistamines are commonly used to treat urticaria; many of these products contain dyes and preservatives known to exacerbate this symptom. To stimulate awareness of this problem a schedule (Table 2) has been compiled of commonly used antihistamines, sub-divided as far as possible into chemical classes, and showing the dyes/preservatives contained. The list is not exhaustive and all companies manufacturing antihistamines may not have been approached. In view of this no implications should be drawn from a drug's omission.

All information was correct as at April 1979 but as formulations are constantly being revised, for the latest information the companies should be approached directly.

TABLE 2. Antihistamines in common use showing the dyes/preservatives contained

| Group | Active Ingredient | Manufacturer | Preparation | Dosage Form | Dye | Preservative |
|---|---|---|---|---|---|---|
| Alkylamine | Brompheniramine | A. & H. Robins | Dimotane | Elixir | Quinoline Yellow | Sodium benzoate |
| | | | | L. A. Tablets | Sunset Yellow F.C.F.<br>Erythrosine | Nil |
| | | | | Tablets | FD & C Yellow No. 6 | Nil |
| | | | | | Tartrazine | Nil |
| | Chlorpheniramine maleate | Allen and Hanbury's Ltd | Piriton | Tablets | Tartrazine | Nil |
| | | | | Spandets | Tartrazine | Nil |
| | | | | Duolet | Sunset Yellow<br>Tartrazine | Nil |
| | | | | Syrup | Tartrazine, Orange G | Nipasept (contains benzoate) |
| | Dimethindene maleate | Zyma | Fenostil retard | Tablets | Tartrazine<br>Sunset Yellow C1 | No salicylate benzoate or related preservative present |
| | Pheniramine maleate | Hoechst | Deneral S.A. | Tablet | Quinoline Yellow<br>Sunset Yellow F.C.F. | Nil |
| Ethanolamine | Diphenylpyraline hydrochloride | Riker | Lergoban | Tablets | Nil | Nil |
| | Diphenhydramine hydrochloride | Parke Davis | Benadryl | Capsules | Erythrosine<br>Patent Blue V | Nil |
| | | | | Cream 2% (discontinued) | Nil | Methylhydroxybenzoate |
| | Diphenhydramine hydrochloride | Parke Davis | Caladryl | Cream | Nil | Propylhydroxybenzoate |
| | Diphenylpyraline hydrochloride | Smith Kline and French | Histryl | Spansule | Amaranth (Pellets)<br>Erythrosine (Shell) | Nil |
| | Diphenylpyraline hydrochloride | Smith Kline and French | Histryl paediatric | Spansule | Amaranth (Pellets)<br>Erythrosine (Shell) | Nil |
| | | | Histryl | Elixir | Tartrazine | Methylparahydroxy-benzoate<br>Propylparahydroxy benzoate |
| Phenothiazine | Mequitazne | Berk | Primalan | Tablet | Nil | Nil |
| Piperazine | Hydroxyzine hydrochloride | Pfizer | Atarax | Tabs. 10 mg. | Dispersed Orange (Anstead 11348) | Nil |
| | | | | Tabs. 25 mg. | Tartrazine<br>Acid Green 56563 | Nil |
| | | | | Syrup | Nil | Sodium benzoate |
| Propylamine | Triprolidine hydrochloride | Wellcome | Actidil | Tablet | Nil | Nil |
| | | | | Elixir | Sunset Yellow F.C.F. | Methylhydroxybenzoate plus sodium benzoate |

TABLE 2. (*cont.*)

| Group | Active Ingredient | Manufacturer | Preparation | Dosage Form | Dye | Preservative |
|---|---|---|---|---|---|---|
| | Triprolidine hydrochloride | Wellcome | Pro-Actidil | Tablet | Nil | Nil |
| Others | Azatadine maleate | Warrick | Optimine | Tablet | Nil | Nil |
| | | | | Syrup | Tartrazine<br>FD & C Yellow 6 | Methyl and propyl parabenzoate |
| | Clemastine hydrogen fumarate | Wander | Tavegil | Tablets | Nil | Nil |
| | | | | Elixir | Nil | Methyl and propyl hydroxybenzoate |
| | Cyproheptadine hydrochloride | Merck Sharp and Dohme | Periactin | Tablers | Nil | Nil |
| | | | | Syrup | Quinoline Yellow E.104 | Sorbic acid |
| | Mebhydrolin napadisylate | Bayer | Fabahistin | Tablet | Yellow Orange S (coat only) | Nil |
| | | | | Suspension | Yellow Orange S | Methyl and propyl hydroxybenzoate |
| | Phenindamine | Roche | Thephorin | Tablets | Dispersed Yellow 1564<br>Titanium Dioxide | Nil |
| | Brompheniramine maleate<br>Phenylephrine hydrochloride<br>Phenylpropanolamine hydrochloride | A. & H. Robins | Dimotapp LA | Tablets | Erythrosine<br>FD & C Blue No. 2<br>Red & Brown Iron Oxides | Nil |
| | As above | A. & H. Robins | Dimotapp | Elixir | FD & C Red No. 2<br>Caramel | Sodium benzoate |
| | Triprolidine hydrochloride<br>Pseudoephedrine hydrochloride | Wellcome | Actifed | Tablets | Nil | Nil |
| | | | | Syrup | Tartrazine | Methylhydroxybenzoate plus sodium benzoate |
| | As above plus codeine | | Actifed compound linctus | Syrup | Amaranth | Methylhydroxybenzoate plus sodium benzoate |
| | Pseudoephedrine hydrochloride<br>Triprolidine hydrochloride<br>Codeine phosphate<br>Guaiphenesin | | Linctus adult expectorant | Syrup | Sunset Yellow F.C.F. | Methylhydroxybenzoate plus sodium benzoate |
| | As above | | Linctifed paediatric expectorant | Syrup | Ponceau 4R | Methylhydroxybenzoate plus sodium benzoate |

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# Do corticosteroids prevent post-herpetic neuralgia?

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## SUMMARY

Forty otherwise healthy patients over 50 years of age with early, severe painful herpes zoster were randomly allocated to two groups for treatment. Twenty patients received prednisolone 40 mg daily with gradual reduction over a period of 4 weeks, whilst the other twenty patients received carbamazepine 100 mg four times daily. Thirteen of the twenty patients (65%) in the carbamazepine treated group developed post-herpetic neuralgia lasting up to 2 years, whilst only three of the twenty prednisolone treated patients (15%) had post-herpetic neuralgia lasting up to 6 months only. Thus the incidence and duration of post-herpetic neuralgia were considerably reduced in the prednisolone treated group. In neither group did disseminated zoster or other complications occur.

One of the most unpleasant and intractable complications of herpes zoster is post-herpetic neuralgia. The incidence of this complication varies with age. It occurs in about 10% of all cases, but is rare under 40 years of age (Burgoon, Burgoon & Baldridge, 1957). In people over 60, more than half are likely to be affected by pain lasting more than a year, as seen in a survey of 916 patients carried out at the Mayo clinic (de Moragas & Kierland, 1957). Post-herpetic neuralgia is especially common after trigeminal herpes zoster, occurring in about 20% of these cases at all ages (de Moragas & Kierland, 1957; *Eye, Ear, Nose, Throat Monthly*, 1969).

As the treatment of established post-herpetic neuralgia is less than satisfactory, prevention of this complication is of great importance. Since the first report of the use of ACTH and cortisone in a case of herpes zoster by Sulzberger *et al.* (1951), a number of uncontrolled reports have been published: five patients of Gelfand (1954); five patients of Appelman (1955); fourteen patients of Sauer (1955); sixteen patients of Elliott (1964); eight patients of Pearce (1973). Only one double-blind study has been reported; this included thirty-five patients by Eaglstein, Katz & Brown (1970).

The earlier reports, indicating dramatic relief of pain within 24 to 36 h, may have been optimistic, but certainly indicated a reduced incidence of post-herpetic neuralgia. This was confirmed by Eaglstein *et al.* (1970) in their double-blind study. In this study the healing time of the skin was comparable in the steroid treated and control groups and there was no correlation between healing time and the presence or absence of post-herpetic neuralgia. Disseminated zoster was not observed in any of these reports.

A contrary view has been expressed: the suggestion that systemic corticosteroid therapy might

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increase the severity of the eruption or lead to dissemination (Merselis, Kaye & Hook, 1964; *Journal of the American Medical Association*, 1964; Juel-Jensen, 1973; *British Medical Journal*, 1979).

## PATIENTS AND METHODS

Forty patients over 50 years of age who were admitted to the dermatological ward with early, severe, painful herpes zoster took part in the trial. Patients with bacterial infection, (other than those secondary to herpes zoster), tuberculosis, diabetes mellitus, peptic ulcer, hypertension, cardio-vascular disease, lymphomas and leukaemia were excluded from the trial. Patients were allocated randomly to either prednisolone treated or carbamazepine treated groups, twenty patients in each group. Carbamazepine was reported to be effective in relieving pain in trigeminal neuralgia, typical and atypical facial pain (Campbell, Graham & Zilkha, 1966, Rasmussen & Riishede, 1970), but had only a minor pain relieving effect in a few cases of post-herpetic neuralgia (Killian & Fromm, 1968). We therefore chose to use it in the control group acting as placebo.

In the prednisolone treated group treatment began with 40 mg prednisolone daily for 10 days, with gradual reduction to nought over the next 3 weeks. In the other group treatment began with carbamazepine 100 mg four times daily for 4 weeks. Topical treatment for both groups was neomycin sulphate 0·25%, gramicidin 0·025% ointment until crusting occurred, thereafter talcum powder. No analgesics were allowed in either group during the acute phase of the illness, but there was free access to analgesics in the post-herpetic neuralgic phase.

After 2 weeks of in-patient treatment, the patients were followed up by one of us (K.K.) in the out-patient clinic at fortnightly intervals until the pain ceased. Pain in the affected area that lasted beyond 2 months from the onset of pain was considered post-herpetic neuralgia. In the absence of objective criteria of pain, only its presence or absence was recorded. The main indication of severity in this condition is its duration which is shown in Tables 1 and 2.

## RESULTS

Twenty patients took part in each group. Male to female ratio was 14:6 in both groups. The average age in the prednisolone treated group was 66·4 years (from 50 to 81), whilst in the carbamazepine treated group the average age was 68·5 years (from 50 to 86). Mean duration of rash before treatment was 5·0 (carbamazepine) and 5·3 (prednisolone) days.

Post-herpetic neuralgia (pain lasting more than 2 months) occurred in thirteen (65%) patients in the carbamazepine treated group and in three (15%) of the prednisolone treated group. All three patients in the prednisolone treated group were over 60 years of age and their pain ceased 4–6 months later. In the carbamazepine group four patients (20%) had pain lasting 1 year or longer, whereas none in the prednisolone treated group had pain exceeding 6 months.

Skin healing was significantly quicker in the prednislone treated group (3·65 weeks) than in the carbamazepine group (5·25 weeks). Clinical details of the two groups are presented in Tables 1 and 2.

## DISCUSSION

Although the cause of post-herpetic neuralgia remains obscure, the pain in the acute phase of the disease is presumed to be due to abnormal pain discharges in the dorsal grey matter of the cord, secondary to inflammation of the segmental dorsal root ganglia. The persistence of pain may be associated with post-inflammatory fibrosis in the root ganglia, or sensory roots, but is perpetuated by central mechanisms. It seems logical to try to inhibit the inflammatory process in the acute phase by the use of

TABLE 1. Prednisolone-treated group

| Case | Age (years) and sex | Site | Duration of eruption before treatment (days) | Time required for skin healing (weeks) | Pain relieved in weeks | Duration of post-herpetic neuralgia (months) |
|---|---|---|---|---|---|---|
| 1 | 72 M | LC5 | 8 | 2 | 3 | — |
| 2 | 80 M | RV1 | 4 | 4 | — | 6 |
| 3 | 63 M | RV1 | 6 | 3 | 1 | — |
| 4 | 51 F | LC6 | 7 | 4 | 4 | — |
| 5 | 50 M | RC7 | 5 | 3 | 2 | — |
| 6 | 53 M | RS1 | 5 | 2 | 2 | — |
| 7 | 76 M | RV2 | 8 | 6 | — | 4 |
| 8 | 71 M | RT5–6 | 2 | 3 | 3 | — |
| 9 | 74 M | RC1 | 4 | 2 | 3 | — |
| 10 | 63 M | RV1 | 3 | 3 | — | 4 |
| 11 | 56 M | LV2 | 3 | 5 | 2 | — |
| 12 | 81 F | LV1 | 2 | 3 | 3 | — |
| 13 | 61 M | RV1 | 3 | 4 | 7 | — |
| 14 | 73 M | RV2 | 5 | 5 | 6 | — |
| 15 | 65 F | LV1 | 6 | 4 | 8 | — |
| 16 | 80 F | LC6 | 5 | 4 | 3 | — |
| 17 | 68 M | LV1 | 7 | 3 | 7 | — |
| 18 | 57 M | LC2 | 7 | 4 | 7 | — |
| 19 | 74 F | RV1 | 8 | 4 | 8 | — |
| 20 | 60 F | RV1 | 8 | 5 | 7 | — |

V = trigeminal; C = cervical; T = thoracic; S = sacral; R = right; L = left.

corticosteroids, since this may avoid the late development of intractable 'central pain'. The definition of post-herpetic neuralgia is arbitrary. Burgoon *et al.* (1957) considered any pain that persisted after the crusts had fallen off the skin lesions to be post-herpetic neuralgia. Sauer (1955) thought it to be pain persisting for 30 days or longer. De Moragas & Kierland (1957) recorded the percentage of patients with pain up to 1 year. Pain which persisted beyond 2 months from its onset was considered post-herpetic neuralgia by Eaglstein *et al.* (1970). We have adopted this last definition. In our series carbamazine did not appear to reduce the pain in the acute phase, or to prevent the occurrence of post-herpetic neuralgia which affected 65% of the patients in this group. The results clearly indicate it was of little or no value. It might be argued that the high incidence of post-herpetic neuralgia and its long duration in the carbamazepine treated group were due to the fact that analgesics were denied to the patients during the acute phase. As analgesics were also denied to the patients in the prednisolone treated group, a valid comparison can still be made between the two groups. In the prednisolone treated group the incidence of post-herpetic neuralgia was only 15%, a low incidence for this age group, particularly as there were more cases of trigeminal herpes zoster in this group (12:7) which one would have expected to produce more cases of post-herpetic neuralgia.

This again supports the claimed efficacy of corticosteroid treatment. The average duration of pain was 5·0 weeks, which, though slightly longer than in previously published reports, is no greater than the control group (5·3), and may indicate the severity of the initial infection.

The three patients with post-herpetic neuralgia were all over 60 years of age and in two of these cases the pain ceased after 4 months and in the third patient disappeared after 6 months. None of these

TABLE 2. Carbamazepine-treated group

| Case | Age (years) and sex | | Site | Duration of eruption before treatment (days) | Time required for skin healing (weeks) | Pain relieved in weeks | Duration of post-herpetic neuralgia (months) |
|---|---|---|---|---|---|---|---|
| 1 | 73 | F | RV1 | 7 | 5 | — | 12 |
| 2 | 81 | M | LC6 | 3 | 8 | — | 8 |
| 3 | 52 | M | RV1 | 3 | 3 | 4 | — |
| 4 | 82 | M | RT2 | 3 | 6 | — | 18 |
| 5 | 86 | F | RV1 | 4 | 3 | — | over 3 |
| 6 | 72 | M | LV1 | 7 | 5 | — | 24 |
| 7 | 69 | M | LC6 | 7 | 6 | 8 | — |
| 8 | 55 | M | LC5 | 3 | 4 | 6 | — |
| 9 | 62 | F | LT10–11 | 12 | 4 | 6 | — |
| 10 | 58 | F | LLu2–3 | 4 | 5 | 5 | — |
| 11 | 79 | F | LLu1–2 | 4 | 6 | — | 6 |
| 12 | 64 | M | RS2 | 3 | 3 | 3 | — |
| 13 | 80 | M | LV1 | 3 | 6 | — | 8 |
| 14 | 63 | M | LC7 | 5 | 4 | — | 8 |
| 15 | 77 | M | LC6 | 2 | 6 | — | 8 |
| 16 | 50 | M | LV1 | 2 | 3 | 5 | — |
| 17 | 74 | F | RV3 | 2 | 4 | — | 6 |
| 18 | 77 | M | RT3–4 | 9 | 4 | — | 5 |
| 19 | 57 | M | LV3 | 10 | 10 | — | 6 |
| 20 | 60 | M | RT1–2 | 7 | 10 | — | 12 |

V = trigeminal; C = cervical; T = thoracic; Lu = lumbar; S = sacral; R = right; L = left.

can therefore be considered as gross, or to compare with those commonly encountered patients whose pain persists for many years and proves intractable. In our series we did not observe any dissemination of the eruption, or other side-effects in the prednisolone treated group. This accords with other reports (Sulzberger *et al.*, 1951; Gelfand, 1954; Appelman, 1955; Sauer, 1955; Elliott, 1964; Eaglstein *et al.*, 1970; Pearce, 1973).

These findings are in sharp contrast with those of Merselis *et al.* (1964), who reported seventeen cases of disseminated herpes zoster in corticosteroid treated patients. Eleven of these patients, however, had suffered from leukaemia or other serious diseases with resultant depressed immune responses. In the remaining six healthy patients the dissemination caused only a mild chickenpox-like eruption.

It is obviously important not to offer corticosteroid treatment for herpes zoster to patients with diminished immune responses, but in our opinion it is justifiable to take a very small risk, e.g. of a mild attack of chicken-pox, in healthy patients over 50 years with herpes zoster, to prevent the high incidence of post-herpetic neuralgia in this age group.

We believe healthy patients over 50 years of age who develop herpes zoster, particularly trigeminal zoster, should be given systemic glucocorticosteroids as soon as possible, preferably within 7 days from the onset. Our experience shows that a moderate dose of prednisolone, 40 mg daily for 10 days, which is gradually tailed off over the following 3 weeks is an effective and safe regime which reduces the occurrence of post-herpetic neuralgia.

It appears that the hazards of systemic glucocorticosteroid treatment in severe herpes zoster have been over-emphasized. A critical review of the literature fails to provide evidence of serious side-effects in patients whose immune responses are not compromised, and there are at least eighty-four published cases (including our own) in which this form of treatment was free of side-effects.

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# The effect of methotrexate and hydroxyurea on neutrophil chemotaxis

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## SUMMARY

Methotrexate depresses motility of neutrophils in chamber membranes, but does not interfere with random motility in tubes. Hydroxyurea has no such effect. The therapeutic response to methotrexate in psoriasis may be due in part to a reduction in migration of neutrophils into the epidermis.

Methotrexate has proved to be an effective treatment for psoriasis. It inhibits the enzyme dihydrofolate reductase and blocks the synthesis of thymidylate, one of the four precursors of DNA, and thereby arrests cell division. In psoriasis, methotrexate is believed to act directly on the rapidly proliferating epidermal cells (Newburger, Weinstein & McCullough, 1978). In generalized pustular psoriasis, the effect of methotrexate is variable but can be dramatic and rapid—so rapid that the response may be due not so much to interference with epidermal cell proliferation, but with neutrophil chemotaxis. We have investigated this possibility by determining the action of methotrexate on neutrophils *in vitro*.

## MATERIAL AND METHODS

For the chemotaxis and random motility tests the concentration of methotrexate used was 2·5 μg/ml of the control medium, this concentration being the upper limit of the range found in serum after therapeutic doses (Halprin, Fukui & Ohkawara, 1971). Folinic acid was used at the same concentration. Hydroxyurea was used at concentrations of 20 μg/ml and 200 μg/ml—levels within and somewhat above those found in the serum after therapeutic doses (Beckloff *et al.*, 1965).

### *Random motility*

Random motility was measured by the microhaematocrit tube technique as described by Miller (1974). The distance migrated upwards by the leading cells after 3 h incubation at 37°C was measured and the result is the mean of measurements in ten tubes.

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### *Neutrophil chemotaxis*

Neutrophil chemotaxis was measured by the double filter technique of Keller *et al.* (1976).

### *Cell separation*

Ten millilitres of heparinized blood (10 iu heparin/ml blood) was added to 1 ml Dextraven 11 (Fison's Ltd), and allowed to sediment for 1 h at room temperature. The supernatant was added to 20 ml Medium 199—modified with Earle's salts, without bicarbonate and glutamine (Flow Labs)—containing Hepes buffer, final concentration 20 mM, and 0·9% glucose. The pH was adjusted to 7·5 with 1 M NaOH. After centrifugation at 400–500 *g* for 15 min, the supernatant was removed and the cells resuspended in the medium to give a concentration of $1 \times 10^6$/ml, counts of viable cells having been determined in a haemocytometer chamber using 0·05% nigrosin dye.

Chemotaxis was measured in the modified Boyden chamber as described by Keller *et al.* (1976). These workers used a double filter technique. However, in an earlier study (Cream & Pole, 1979) it was shown that there is no advantage in using two filters and a single Sartorius cellulose nitrate filter, 3 μm pore size, was therefore employed. The chemotactic agent was 2 vol. of 0·5% casein (Sigma Ltd) in Medium 199 plus 1 vol. of AB serum. The chemotactic agent was added to the lower well and 0·25 ml cell suspension to the upper. Chambers were set up in duplicate and incubated for 110 min at 37°C, before the filters were fixed in 80% ethanol–20% butanol mixture for 5 min. The filters were stained with Harris's haematoxylin for 1 min, washed and then dehydrated successively in 70% propanol, 95% propanol and 80% ethanol–20% butanol mixture. After clearing in xylene, the filters were mounted in DPX mountant. Using a 1 $cm^2$ graticule eyepiece, cell counts were performed in five high power fields (×400) over the lower surface of the 3 μm filter and the sum of the high power field counts was taken as the chemotactic index.

Methotrexate, folinic acid and hydroxyurea were added to the cell suspensions in appropriate concentration and so were present in the chamber during chemotaxis. The effect of preincubation with methotrexate and resuspension in methotrexate-free medium was also determined. Cells prepared as above were incubated for 30 min at 37°C in methotrexate 2·5 μg/ml. After centrifugation at 400–500 *g* for 15 min the supernatant was removed and the pellets resuspended to give approximately 5 ml suspensions in either medium or methotrexate 2·5 μg/ml, with $10^6$ cells/ml.

## RESULTS

### *Random motility in microhaematocrit tubes*

Table 1 shows the results for random motility in the control medium, in the presence of methotrexate and in the presence of hydroxyurea. The difference between the means in the control medium and in the presence of methotrexate was not significant. Nor were there significant differences between random motility in control medium and in the two concentrations of hydroxyurea.

### *Neutrophil chemotaxis*

The chemotactic indices in the control medium, in the presence of methotrexate, or methotrexate and folinic acid together are shown in Table 2. The chemotactic indices for folinic acid alone are given in Table 3. Methotrexate depressed chemotaxis, the difference between the means for chemotaxis in the control medium and in the presence of methotrexate being 41·865, a significant difference ($t = 4{\cdot}408$; $P < 0{\cdot}001$). Folinic acid alone did not significantly depress chemotaxis, the difference between chemotaxis in the control medium and in the presence of folinic acid being 29·9 ($t = 2{\cdot}005$; $P > 0{\cdot}05$). Folinic acid counteracted the depressive effect of methotrexate in that there was no significant difference between the means of chemotaxis in the control medium and in the presence of both methotrexate and folinic acid (difference between the means = 7·44, $t = 0{\cdot}5013$; $P > 0{\cdot}05$).

TABLE 1. The effect of methotrexate and hydroxyurea on random motility in micro-haematocrit tubes

| | Control medium | Methotrexate (2·5 μg/ml) | Control medium | Hydroxyurea | |
|---|---|---|---|---|---|
| | | | | (20 μg/ml) | (200 μg/ml) |
| | 6·3 | 3·7 | 3·3 | 3·5 | 2·9 |
| | 5·5 | 3·8 | 4·5 | 5·1 | 4·2 |
| | 4·7 | 5·1 | 4·7 | 4·1 | 2·9 |
| | 4·0 | 4·3 | 4·1 | 3·6 | 3·3 |
| | 2·5 | 2·6 | 4·3 | 4·0 | 4·0 |
| | 4·0 | 4·1 | 3·4 | 4·3 | 3·2 |
| | 2·8 | 2·3 | | | |
| Mean | 4·26 | 3·7 | 4·05 | 4·1 | 3·4 |
| S.d. | 1·34 | 0·97 | 0·578 | 0·576 | 0·556 |

| | Difference between means | t | *P* |
|---|---|---|---|
| Control and methotrexate | 0·56 | 1·76 | n.s. |
| Control and hydroxyurea (20 μg/ml) | 0·05 | 0·199 | n.s. |
| Control and hydroxyurea (200 μg/ml) | 0·7 | 2·548 | n.s. |

TABLE 2. The effects of methotrexate and folinic acid on chemotactic index

| | Control | Methotrexate (2·5 μg/ml) | Methotrexate (2·5 μg/ml) and folinic acid (2·5 μg/ml) |
|---|---|---|---|
| | 254 | 196·5 | 367 |
| | 209·5 | 192 | 244·5 |
| | 210 | 206 | 298 |
| | 334 | 223·5 | 246 |
| | 335 | 203 | 258·5 |
| | 84 | 37 | 62·5 |
| | 124·5 | 115 | 107·5 |
| | 116·5 | 75·5 | 106·5 |
| | 103 | 88·5 | 71 |
| | 163·5 | 131·5 | 135·5 |
| | 109·5 | 71·5 | 90 |
| | 207 | 187·5 | 216 |
| | 180·5 | 158 | 175·5 |
| | 304 | 212 | 283 |
| | 152·5 | 129 | 158·5 |
| | 186 | 175·5 | 149·5 |
| Mean | 192·5 | 150·13 | 185·5 |
| S.d. | 80·43 | 58·6 | 90·449 |

| | Difference between means | t | *P* |
|---|---|---|---|
| Control and methotrexate | 41·865 | 4·408 | < 0·001 |
| Control and methotrexate and folinic acid | 7·44 | 0·5013 | > 0·05 (n.s.) |

TABLE 3. The effect of folinic acid on chemotactic index

| | Control | Folinic acid (2·5 μg/ml) |
|---|---|---|
| | 124·5 | 116·5 |
| | 116·5 | 88·5 |
| | 103 | 67 |
| | 163·5 | 141 |
| | 109·5 | 81 |
| | 207 | 200 |
| | 180·5 | 151·8 |
| | 304 | 149 |
| | 152·5 | 177·5 |
| | 186 | 170 |
| Mean | 164·7 | 134·8 |
| S.d. | 60·406 | 43·706 |
| Difference between means | | 29·9 |
| t | | 2·005 |
| *P* | | >0·05 (n.s.) |

TABLE 4. The effect of hydroxyurea on chemotactic index

| | Control | Hydroxyurea (20 μg/ml) | Hydroxyurea (200 μg/ml) |
|---|---|---|---|
| | 85·5 | 118·0 | 96·5 |
| | 132·0 | 91·0 | 92·5 |
| | 126·0 | 100·5 | 74·0 |
| | 51·5 | 56·5 | 27·5 |
| | 108·0 | 109·5 | 70·5 |
| | 86·5 | 129·5 | 94·0 |
| | 123·0 | 101·5 | 97·5 |
| | 72·0 | 99·0 | 150·5 |
| | 89·0 | 107·5 | 81·5 |
| | 134·0 | 88·0 | 68·5 |
| Mean | 100·76 | 100·1 | 85·3 |
| S.d. | 28·07 | 19·6 | 30·97 |

| | Difference between means | t | *P* |
|---|---|---|---|
| Control and hydroxyurea (20 μg/ml) | 0·66 | 0·066 | n.s. |
| Control and hydroxyurea (200 μg/ml) | 15·46 | 1·189 | n.s. |

The chemotactic indices in the presence of hydroxyurea are shown in Table 4. No significant depression of chemotaxis was seen at hydroxyurea concentrations of 20 μg and 200 μg/ml.

The effect on the chemotactic index of preincubation with methotrexate for 30 min at 37°C and subsequent resuspension in methotrexate-free medium is shown in Table 5. Chemotaxis remained depressed when the cells incubated in methotrexate were resuspended in the control medium.

TABLE 5. The effect on chemotactic index of preincubation with methotrexate and subsequent resuspension in methotrexate-free medium

| | Control | Methotrexate (2·5 µg/ml) | Preincubated in methotrexate and resuspended in medium |
|---|---|---|---|
| | 203·5 | 115 | 113 |
| | 191·0 | 138·5 | 136·5 |
| | 124·5 | 98·5 | 92·5 |
| | 168·0 | 136·0 | 128·5 |
| | 151·0 | 149·5 | 138 |
| | 149·5 | 120·0 | 139 |
| Mean | 164·58 | 126·25 | 124·58 |
| S.d. | 29·13 | 18·55 | 18·486 |

| | Difference between means | t | *P* |
|---|---|---|---|
| Control and methotrexate (2·5 µg/ml) | 38·33 | 3·185 | <0·05 |
| Methotrexate (2·5 µg/ml) and preincubated cells | 0·38 | 1·66 | n.s. |

TABLE 6. Relationship between concentration of methotrexate and depression of chemotaxis

| Methotrexate (µg/ml) | 1·25 | 2·5 | 5 | 10 | 20 |
|---|---|---|---|---|---|
| Chemotaxis (% of control) | | | | | |
| Mean | 76·4 | 61·8 | 75·6 | 65·4 | 53·5 |
| S.d. | 26·5 | 19·2 | 19·9 | 11·3 | 16·6 |

Table 6 shows the relationship between concentration of methotrexate and depression of chemotaxis. The figures given are the means and standard deviations for three experiments at each concentration.

## DISCUSSION

Methotrexate has proved to be a valuable drug in the treatment of psoriasis. It inhibits dihydrofolate reductase and one of the effects is to block the synthesis of thymidylate—a precursor of DNA. Cell division is thus arrested. The site of action of methotrexate in psoriasis is thought to be the rapidly proliferating epidermal cell in the psoriatic plaque, since injection of methotrexate into a plaque is followed by a dose related decrease in mitotic activity and the appearance of damaged cells (Newburger *et al.*, 1978).

Clinical improvement in psoriatic plaques after administration of methotrexate is usually slow, as might be expected if the sole action of methotrexate was to depress epidermal proliferation. However, the drug has other actions as judged by its efficacy in arthritis and it has been shown to possess anti-inflammatory properties (Hurd, 1973). In psoriasis a possible action on polymorphs is suggested by: (1) the sometimes immediate response to methotrexate in generalized pustular psoriasis (Ryan &

Baker, 1969) and (2) the prominence of polymorphs in the epidermis in generalized pustular psoriasis and their presence at an early stage in less active forms of psoriasis. It is relevant to note here that psoriatic plaques contain increased amounts of serine proteinase which activates complement and induces polymorph accumulation (Lazarus, Yost & Thomas, 1977).

Our results have demonstrated that *in vitro* methotrexate does have an effect on polymorphs in that it depresses motility in chamber membranes. Random motility in tubes was unaffected. Hydroxyurea did not interfere with chemotaxis or random motility.

Granulocytes take up methotrexate by diffusion and by a saturable temperature sensitive transport process (Kessel, Hall & DeWayne, 1968). The precise mode of action of methotrexate on polymorphs is not apparent. The fact that motility is depressed after preincubation with methotrexate and subsequent resuspension in methotrexate-free solution indicates that the action of methotrexate is not due to any effect the drug may have on pH or ionic concentrations in the medium. The known metabolic consequences of methotrexate include inhibition of thymidylate synthetase and inhibition of synthesis of purines, RNA and proteins (Flaxman *et al.*, 1977). A selective depression of chemotaxis without an effect on random motility in tubes has been demonstrated after pre-incubation with cyclic AMP and after inhibition of glycolysis by iodacetate or sodium fluoride. Interference with oxidative metabolism does not interfere with chemotaxis or random motility in tubes (Miller, 1974).

Folinic acid apparently reversed the action of methotrexate. However, in our experiments this could simply have been due to the fact that folinic acid competitively inhibits the uptake of methotrexate by granulocytes (Kessel *et al.*, 1968).

We have shown that methotrexate has a depressive action on neutrophil motility. In the absence of results for random motility in chamber membranes, we are not able to say whether this depression is due to an effect on random motility or on chemotaxis, and in this respect the results for the tube random motility cannot be taken into account since random motility in tubes is not necessarily comparable with that in membranes (Miller, 1974).

The *in vitro* action of methotrexate on neutrophils raises the possibility that in psoriasis the improvement after methotrexate may be due in part to a reduction in the numbers of neutrophils migrating into the epidermis. If this is so, other drugs which interfere with neutrophil chemotaxis may have a place in the management of generalized pustular psoriasis. Fusidic acid, tetracycline and rifampicin are amongst the antibiotics which—in concentrations comparable to therapeutic blood levels—markedly depress neutroplil migration *in vitro* (Forsgren & Schmeling, 1977; Esterley, Furey & Flanagan, 1978). The effect of these drugs in generalized pustular psoriasis should be assessed.

## ACKNOWLEDGMENTS

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# The effect of steroid and dithranol therapy on cyclic nucleotides in psoriatic epidermis

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## SUMMARY

Absolute values of both cAMP and cGMP levels were measured in the involved and uninvolved skin of psoriatic patients, and also the effect of topical therapy on these levels in the involved skin was studied. The mean cGMP level in the untreated psoriatic plaque was increased by 300% compared to the non-involved skin (which did not differ from normal skin), but no significant difference in cAMP levels was found. Epidermal stripping of uninvolved skin, which stimulates cell proliferation, did not change the cGMP level.

Treatment of the psoriasis with dithranol caused the cGMP levels to return to normal, but a potent topical glucocorticoid, in contrast, produced no such decrease. This may imply that the two drugs act at different levels in suppressing cell replication, and dithranol may be a useful tool for the further investigation of cyclic nucleotide metabolism.

Abnormalities in cyclic nucleotide metabolism may be important in the pathogenesis of psoriasis. Early reports suggested that cyclic AMP levels (cAMP) were decreased in the involved skin of psoriatic patients (Voorhees *et al.*, 1972), but later work failed to confirm this (Harkonen, Hopsu-Havu & Raij, 1974; Yoshikawa *et al.*, 1975b) and it now seems that cyclic GMP (cGMP) levels may be more important than cAMP levels in regulating epidermal cell division (Halprin, 1976). Cyclic GMP is technically difficult to estimate, and only one group has reported absolute values of cGMP levels in the psoriatic epidermis (Marcelo *et al.*, 1979). We have now measured absolute levels of both cAMP and cGMP in the involved and uninvolved skin of psoriatic patients, and we have also studied the effect of dithranol therapy and topical steroid therapy on these levels in the involved skin.

## MATERIALS AND METHODS

Skin samples were obtained from both the involved and the uninvolved skin of the back of fourteen untreated psoriatic patients, and from seven normal controls, using a Castroviejo electro-keratotome

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with a setting of 0·4 mm for the involved skin and 0·2 mm for the uninvolved skin. The skin was cleaned prior to biopsy by gentle swabbing with 0·02% aqueous chlorhexidine. The biopsy technique causes very little pain, and no anaesthetic was used, since local anaesthetic injection could affect levels of cyclic nucleotides (Yoshikawa *et al.*, 1975a).

In a subsequent experiment, samples from different but clinically similar patches of involved skin were obtained in the same way from nine psoriatic patients before and after twice daily treatment with topical 0·05% clobetasol propionate (Dermovate) ointment and from seven psoriatic patients before and after the daily application of dithranol 0·1–0·25% in Lassar's paste. In each case the treatment was applied for 5 days, and the lesions were clinically improved but not completely clear at the time of the second biopsy.

We also obtained skin from a further eight patients with psoriasis before and after epidermal stripping. Uninvolved skin was stripped ×30 with Scotch tape until a red glazed appearance was produced. Samples were taken 72 h later from the stripped skin and from the contralateral uninvolved unstripped skin.

All the skin samples were immediately snap-frozen in liquid nitrogen. They were transported to the laboratory and extracted within 30 min of collection. A portion of each specimen was also examined histologically, and those samples which did not include the epidermal basal layer or which included an excess of dermis were discarded. The frozen tissue was then weighed and dropped into 0·5 ml of 6 mM theophylline at 100°C and boiled for 10 min. The denatured tissue was fully homogenized and after addition of 0·5 ml of acid ethanol (1% N HCl in absolute alcohol), it was stored overnight at −20°C. Samples were then centrifuged at 1500 *g* for 10 min and the supernatants decanted. Each precipitate was washed with 0·5 ml of acid alcohol and after respinning the corresponding supernatants were pooled. The extracts were taken to dryness at 50°C, the dried residue was resuspended in 0·5 ml of 50 mM Tris HCl buffer (pH 7·5) and then assayed for cyclic nucleotide content. The cAMP levels were assayed by a protein-binding method (Brown *et al.*, 1971) and cGMP levels were measured by a radio-immunoassay (Albano, Bhoola & Harvey, 1976).

Nucleotide estimations were performed in triplicate and the results expressed as pmol of cyclic nucleotide/mg wet wt. of tissue.

TABLE 1. Cyclic nucleotide levels in normal epidermis and in psoriatic epidermis

| | | cAMP (pmol/mg wet weight) | cGMP (pmol/mg wet weight) |
|---|---|---|---|
| Skin source | No. | Mean ± s.e. | Mean ± s.e. |
| Normal | 7 | 0·67 ± 0·23 | 0·008 ± 0·005 |
| Uninvolved psoriasis | 14 | 0·88 ± 0·27 | 0·007 ± 0·002 |
| Involved psoriasis (untreated) | 14 | 0·70 ± 0·13 | 0·024 ± 0·003 |
| Involved psoriasis: | | | |
| Before dithranol | 7 | 1·25 ± 0·19 | 0·032 ± 0·009 |
| After dithranol | 7 | 0·87 ± 0·11 | 0·005 ± 0·003 |
| Involved psoriasis: | | | |
| Before Dermovate | 9 | 0·68 ± 0·11 | 0·015 ± 0·004 |
| After Dermovate | 9 | 0·66 ± 0·07 | 0·013 ± 0·002 |
| Uninvolved psoriasis: | | | |
| Before stripping | 8 | 0·94 ± 0·17 | 0·004 ± 0·002 |
| After stripping | 8 | 1·05 ± 0·16 | 0·003 ± 0·001 |

## RESULTS

The results of the cyclic nucleotide assays are shown in Table 1.

The cAMP concentrations in involved (untreated) and uninvolved psoriatic epidermis did not differ significantly from the level found in normal epidermis, but the mean cGMP concentration in involved psoriatic epidermis (0·024 pmol/mg wet wt.) was significantly greater than the levels found in the uninvolved skin (0·007 pmol/mg wet wt., $P<0·01$), and in the normal epidermis (0·008 pmol/mg wet wt., $P<0·01$). The ratio cAMP/cGMP was greatly reduced in the psoriatic plaque compared to both uninvolved psoriatic skin and normal skin (Fig. 1).

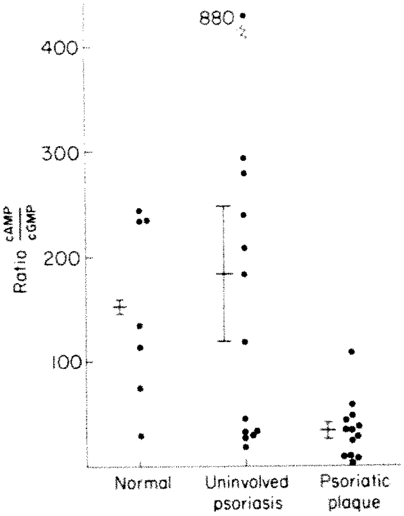

FIGURE 1. The ratio cAMP/cGMP in keratotomed epidermis from the skin of normal subjects compared with the uninvolved and lesional skin of patients with psoriasis. Bars represent mean ± s.e.

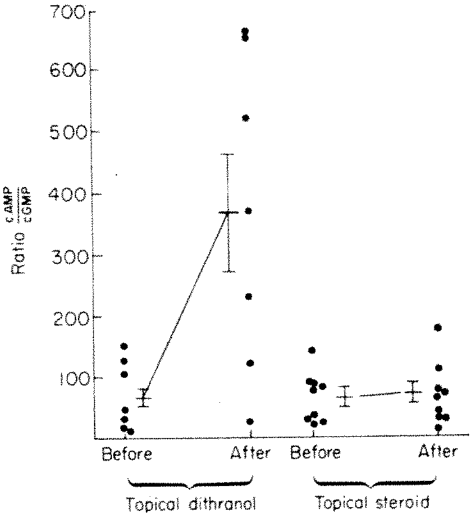

FIGURE 2. The ratio cAMP/cGMP in keratotomed epidermis from psoriatic plaques before and after 5 days treatment with either dithranol or clobetasol propionate. Bars represent mean ± s.e.

Treatment of the psoriatic plaques with dithranol caused a significant decrease in cGMP concentration (from 0·032 to 0·005 pmol/mg wet wt., $P<0{\cdot}05$), but the effect on cAMP was not significant. By contrast, the lesions treated with clobetasol propionate showed no significant change in either the cAMP or cGMP levels (Fig. 2).

Epidermal stripping 72 h prior to the sampling of uninvolved psoriatic skin had no significant effect on either cAMP or cGMP levels.

## DISCUSSION

We have found the mean cGMP concentration in the untreated lesional epidermis of patients with psoriasis to be increased by about 300% compared to the non-involved epidermis in the same patients, and this confirms the significant but smaller increase recently reported by the Michigan group (Marcelo *et al.*, 1979). There was reasonably good correspondence between the absolute figures reported by the two groups. Our initial figure of 0·024 pmol/mg wet wt. for lesional skin corresponds well with their figure of 0·018 pmol/mg wet wt., and in our subsequent experiments in two other groups of psoriatic patients we found mean values for lesional skin of 0·015 and 0·032 pmol/mg wet wt. respectively. Their mean value for the cGMP concentration in the epidermis of normal subjects was somewhat greater than ours (0·015 compared to our 0·008 pmol/mg wet wt.), but both groups agree that the mean cGMP level in non-involved psoriatic epidermis does not differ significantly from that in normal skin.

We also agree with the recent conclusion of Marcelo *et al.* (1979) and with the previous reports of Yoshikawa *et al.* (1975b) and Halprin *et al.* (1975) that cAMP levels in lesional skin do not differ significantly from those in non-involved skin. We could not, however, confirm the finding of the Michigan group that the cAMP content of all psoriatic epidermis is decreased compared to the normal epidermis. Our figure for cAMP level in the normal epidermis (0·67 pmol/mg wet wt.) corresponds closely to their figure (0·81 pmol/mg wet wt.) but we found no decrease in cAMP concentration in the psoriatic tissue.

The effect of treatment on the cyclic nucleotide levels does not appear to have been previously reported. We found that dithranol applied to the psoriatic plaque caused a marked decrease in the cGMP level, which returned to the normal value. The application of a potent topical steroid (clobetasol propionate), however, produced no significant fall in cGMP even though the plaques showed a clinical improvement, and this may imply that the two drugs exert their beneficial effects in psoriasis by different mechanisms.

It seems unlikely that the increased levels of cGMP we observed in lesional psoriatic skin were secondary to increased epidermal proliferation, since epidermal stripping of uninvolved skin of psoriatic subjects caused no subsequent rise in cGMP.

Our data thus support the suggestion that increased cyclic GMP production is involved in the formation of a psoriatic plaque, but the cause of this increase is unknown. Abnormalities in arachidonic acid and prostaglandin metabolism have been reported in psoriasis (Hammerstrom *et al.*, 1975; Plummer *et al.* 1978) and further studies of the relationship between prostaglandins and cyclic nucleotides are required. Dithranol may be a useful tool for uncovering this relationship.

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# Limited application period for dithranol in psoriasis

## PRELIMINARY REPORT ON PENETRATION AND CLINICAL EFFICACY

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SUMMARY

This preliminary study suggests that a 1 h application of dithranol may be effective in the treatment of psoriasis. It is conceivable that short application periods for other topically applied agents, e.g. corticosteroids, may reduce the side-effects in non-affected skin, while maintaining the therapeutic benefit in diseased areas.

Dithranol is established as a highly effective treatment for psoriasis (Ingram, 1953; Farber & Harris, 1970). The disadvantages in its use are the staining of clothing and linen, irritation, staining of skin and long application time.

Previous studies on the penetration kinetics of almost forty different topically applied drugs revealed that most of them reach maximal concentration in normal skin after 30–100 min (Schaefer, Zesch & Stüttgen, 1977) or sooner in stripped skin. Furthermore, studies done in Berlin (Kammerau, Zesch & Schaefer, 1975; Schalla, 1979) and Stanford (Goldberg *et al.*, unpublished results) using tritiated dithranol showed penetration of the drug through the full thickness of the epidermis in 100 min or less.

Thus, the idea was evolved that by shortening the application period, i.e. by removing the surplus of dithranol ointment from the skin surface after 1 h, the side-effects could be reduced without impeding the therapeutic efficiency.

Two questions have to be answered to test this working hypothesis: (1) Is the penetration of dithranol into diseased skin still sufficient, if it is interrupted after a short application time? (2) Can an anti-psoriatic effect still be demonstrated under these conditions?

In order to answer these questions, the following experiments were performed.

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## PENETRATION STUDY

Tritium labelled 0·1% dithranol in vaseline was applied to human skin *in vitro* (Material and Methods, see Schaefer *et al.*, 1980). In half of the experiments the horny layer was removed before application to mimic diseased skin. In one experiment the ointment was left on the skin for 1000 min; in another the surplus was removed after 30 min by washing with a 10% soap solution (Forlan); in a third the penetration was terminated after 30 min. Skin specimens from one donor and the same area (ventral cadaver skin) were used for all six experiments. The results are shown in Table 1.

TABLE 1. Penetration of dithranol 0·1% in vaseline into human skin *in vitro* relative to horny layer status and application period. (Quantities are given in $\mu g/cm^2 \times$ thickness of the respective layer)

| | Epidermis | | Upper dermis | |
|---|---|---|---|---|
| Penetration period (min) | Normal skin | Horny layer removed | Normal skin | Horny layer removed |
| 30 | 6 | 325 | 1 | 12 |
| 30–1000* | 5 | 160 | 2 | 27 |
| 1000 min | 110 | 500 | 14 | 69 |

* Surplus removed after 30 min, then penetration continued.

The following conclusions can be drawn. In normal skin the onset of penetration of dithranol (from this ointment) is slow; ca 20 times more drug enters the epidermis, ca 5 times more drug the upper dermis after a 1000 min application period relative to a 30 min application period. When the horny layer barrier is disturbed, this difference decreases to ca 1·5 times in the epidermis and ca 5 times in the upper dermis. The results in the second experiment are similar to those in the first. The removal of the surplus of dithranol by soap favours the diffusion of the drug into the dermis, when the penetration continues.

Thus, by removal of the surplus the concentration is decreased by a factor of 2–3 in 'diseased' skin, whereas in the normal skin it is decreased by a factor of 7–20. In other words, the margin between the undesirable amount of drug in normal skin (responsible for side-effects) and the therapeutically active drug in disturbed areas is increased.

## CLINICAL STUDY

In order to make use of the fact that in normal skin the penetration is slower and lower, i.e. to reduce side-effects, a preliminary clinical trial was planned with 1% dithranol and a 1 h application period. The concentration of 1% instead of 0·1% was used in order to compensate for the decrease of penetration by the shortened application period (according to the rule of thumb that a tenfold increase in the applied amount causes a roughly threefold increase in penetration rates). One per cent salicylic acid was added to stabilize the dithranol (Ingram, 1956; Ponec-Waelsch & Hulsebosch, 1974).

Thirteen patients with stable plaque psoriasis were treated with 1% dithranol and 1% salicylic acid in vaseline for 1 h daily. The ointment was rubbed in thoroughly to a single plaque only, while patients continued to treat other lesions with their previously used topical medication (steroids and tar). After 1 h the dithranol was removed from the single lesion by washing with soap and water. After 4–6 weeks the treated plaque was assessed.

TABLE 2. Clinical results of 1 h treatment of psoriatic plaques with dithranol

| No. of patients | Results |
|---|---|
| 3 | Cleared (plaque resolved) |
| 4 | Lesion flat, but some erythema (plaque resolved—residual erythema) |
| 4 | Lesion improved but still raised (plaque less elevated with patchy resolution) |
| 2 | Lesion still raised (little change) |

Table 2 shows the results of single plaques treated with 1% dithranol at the end of 4–6 weeks. Plaques treated with the regular regimen showed no change.

Patients were satisfied with the ointment and found that the 1 h application time was convenient for use at home. The limited application time encouraged greater patient compliance and hence stricter adherence to a less rigorous regimen.

Although this group of patients were assessed at 4–6 weeks of treatment, two patients have shown continued resolution of their lesions up to 4 months later.

As is usual with the use of dithranol, there was some redness and pigmentation of the surrounding skin. However, there was no case of a patient who experienced side-effects so that he/she stopped the treatment.

Surprisingly, the staining of the plaque was noticeable as with longer application periods of dithranol. The application of 1% dithranol for 1 h every day seems to be equivalent clinically to application of lower strength dithranol for 8–10 h. This could facilitate the treatment of psoriatics as outpatients or in day care centres. A controlled clinical trial is now in progress.

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# Treatment of psoriasis and atopic dermatitis with halcinonide cream applied once and three times daily

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## SUMMARY

In a double-blind multi-centre study, comprising ninety-five patients with psoriasis and atopic dermatitis, 0·1% halcinonide cream applied once daily was equally as effective as the cream applied three times daily. However, the onset of action was more rapid when the cream was applied three times daily. In a control study once daily application of 0·1% halcinonide cream was found to be superior to the vehicle alone in the treatment of forty patients with the same diseases.

By tradition topical steroids are applied two to four times daily. These recommendations have been derived empirically, but there are few if any pharmacokinetic or clinical data to support them. Our study was, therefore, designed to evaluate once daily application of a halcinonide cream versus three times daily in the treatment of psoriasis and atopic dermatitis. As a control the vehicle alone was compared with once daily application of the active cream.

## MATERIAL AND METHODS

Fifty-one patients with chronic nummular psoriasis were included in the study. Of these sixteen were assigned to a study in which 0·1% halcinonide cream applied once daily was compared with placebo. In the remaining thirty-five patients once daily application of 0·1% halcinonide cream was compared with three times daily application of the same cream. There were twenty-seven males and twenty-four females. Their ages varied between 19 and 73 years with an average of 39·8 years. Eighty-four patients with atopic dermatitis were studied. Of these twenty-four were assigned to the comparison with placebo, and in the remaining sixty once daily application was compared with three times daily application of 0·1% halcinonide cream. There were thirty-eight males and forty-six females. Their ages varied between 1 and 68 years, the average being 22·6 years.

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For both materials patients with symmetrical lesions of equal severity were chosen for comparison of the different treatment modalities. The study was run in a double-blind randomized fashion. Each patient received two sets of tubes marked left and right. Each set consisted of three tubes marked morning, afternoon and evening plus a code number. In the once daily group only the tube marked evening contained the active ingredient (0·1% halcinonide) while the other two tubes contained the vehicle only. In the placebo group all three tubes contained the vehicle only, and in the three times daily group all tubes contained 0·1% halcinonide. No concomitant treatment was allowed.

The patients were observed once weekly for 3 weeks. However, if the lesions on one or both sides were completely healed at one of the earlier weekly assessments, the patient was withdrawn from the study. At the weekly assessments the comparative response was noted as equal, slightly superior or markedly superior. The therapeutic response for each side compared to pre-treatment condition was noted as less than 25%, 25–49%, 50–74% and 75–100% improvement. In the overall evaluation also the comparative response was noted as above, and the therapeutic response was noted as poor, fair, good or excellent. In the statistical evaluation $\chi^2$-test was used.

## RESULTS

From Table 1 it is evident that once daily application of 0·1% halcinonide is superior to placebo. The difference is highly significant ($P<0{\cdot}001$). With the exception of two psoriatic patients all placebo responders were found in the patients with atopic dermatitis.

The results of the study where once daily application of halcinonide was compared with three times daily application are summarized in Tables 2 and 3. When halcinonide is applied three times daily the onset of action is more rapid than when it is applied once daily (Table 2). At the end of treatment there is no statistical difference with regard to good and excellent overall response (Table 3). At that time the response was equal on both sides of the body in 74% of the patients.

TABLE 1. Overall evaluation of therapeutic response: halcinonide once daily versus placebo

| Overall response | Halcinonide (once daily) | Placebo |
|---|---|---|
| Excellent | 18 | 7 |
| Good | 18 | 12 |
| Fair | 3 | 5 |
| Poor | 1 | 16 |

TABLE 2. Comparable clinical response: halcinonide once daily versus three times daily

| | | Halcinonide superior (once daily) | | Halcinonide superior (three times daily) | | |
|---|---|---|---|---|---|---|
| Evaluation | N | Markedly | Slightly | Markedly | Slightly | Both equal |
| Week 1 | 95 | 3 | 9 | 5 | 27 | 51 |
| Week 2 | 91 | 2 | 10 | 10 | 10 | 59 |
| Week 3 | 83 | 1 | 6 | 9 | 7 | 60 |

TABLE 3. Overall evaluation of therapeutic response: halcinonide once daily versus three times daily

| Overall response | Halcinonide (once daily) | Halcinonide (three times daily) |
|---|---|---|
| Excellent | 36 | 46 |
| Good | 40 | 35 |
| Fair | 15 | 11 |
| Poor | 4 | 3 |

Side-effects were noted in fifteen of the patients and only in those with atopic dermatitis. The side-effects were in general mild and consisted usually of erythema, burning and pruritus. Treatment was withdrawn in three patients: in one case because of increased pruritus; in two cases because of increased erythema.

## DISCUSSION

Halcinonide is a well-known potent corticosteroid and it is thus logical that it was found to be superior to the placebo in this study also. The fact that there were many placebo responders among the patients with atopic dermatitis is probably due to the soothing action of the vehicle alone.

That once daily application is equally as effective as three times daily application is not really surprising. The absorption of corticosteroids through human skin is a comparatively slow process, a phenomenon that has been referred to as a reservoir effect by Vickers (1963) and Feldmann & Maibach (1968). It has also been shown that very frequent applications are of no greater value.

Eaglestein, Farzad & Caplan (1974) thus showed that six applications daily was not superior to three applications per day of another steroid. Du Vivier & Stoughton (1975) described acute tolerance or tachyphylaxis to topical corticosteroids which is of interest in this connection. However, if the clinical response is equal, the absorption of effective steroid should be the same and also the degree of tachyphylaxis. If this is true it is difficult to explain the more rapid onset of action when halcinonide is applied three times daily. On the other hand, the difference is small and may be of no importance.

The emollient effect of the vehicle cannot be altogether disregarded when comparing the results of the two modes of application. However, because of the double-blind design of the study it was not possible to avoid this possibly disturbing factor in the assessment. Anyway, once a day application of a steroid—with or without additional application of an emollient cream—will have the advantage that it will be considerably cheaper. If the emollient cream is not used, once daily application of the steroid will be much more convenient with better patient compliance as a result.

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# Plasma zinc in psoriatic in-patients treated with local zinc applications

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## SUMMARY

Zinc is a major component of many local skin applications. An investigation into its systemic absorption in thirty-one psoriatic hospital in-patients is reported. Plasma zinc, copper and whole blood delta-aminolaevulinic acid dehydratase were followed before and during in-patient treatment over 14 days. No significant alteration in these parameters was detected.

Significantly reduced plasma zinc levels were found in the patients compared to a matched control population.

Zinc compounds form a major component of many topical skin preparations; over 100 of these are listed in Martindale (1977).

Zinc oxide, the most frequently used compound, is used as an inert vehicle with mild astringent and local drying properties. It is a component of many substances which are applied repeatedly to extensive areas of diseased skin in chronic disorders such as psoriasis and eczema.

However, the biological importance of zinc itself is now well established, including a role in the synthesis of DNA, RNA and collagen (Henkin, 1974; Hallmans, 1978b). It may therefore be an important factor in systems undergoing rapid cell division such as healing wounds or psoriatic epidermis (Pories *et al.*, 1967).

The effects of oral zinc supplementation in wound healing and in skin diseases such as leg ulceration have been well studied (Pories *et al.*, 1967), but the possibility that local application of zinc compounds to diseased skin might have a direct therapeutic effect through percutaneous absorption has not been widely explored. It is well recognized that systemic absorption of topically applied steroid preparations occurs in a variety of skin disorders, to such a degree as to cause toxicity. Local salicylic acid applications have also recently been associated with systemic toxicity (Davies, Vella Briffa & Greaves, 1979).

It seems likely also that some absorption of zinc oxide occurs through abraded skin, and Hallmans (1978a) has found a significant increase in serum zinc concentration in rats treated with zinc oxide

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applications to excisional wounds. Hallmans (1978b) also studied serum zinc in eight patients with extensive burns, treated with zinc tape locally, and found significant elevation of serum zinc, reaching a peak during the third week of treatment.

The authors, therefore, decided to investigate plasma zinc, plasma copper and blood delta-aminolaevulinic acid dehydratase (δ-ALA.D, a haem enzyme which is known to be zinc dependent in physiological concentrations in man (Finelli *et al.*, 1974; Meredith & Moore, 1978)) in psoriatic in-patients during treatment with compounds containing zinc oxide, a salt which is insoluble in aqueous solutions. A change in these parameters might indicate systemic zinc absorption.

Analyses of the ointments applied to each patient were also undertaken to provide a quantitative estimate of the zinc applied over the 2-week study period.

## PATIENTS AND METHODS

Thirty-one patients with chronic psoriasis, previously treated at home, were investigated. The results from one patient were later discounted due to the discovery of lead exposure. Eight patients had had tar or dithranol applications containing zinc at home during the 6 months prior to admission, but their mean plasma zinc was not different from the others.

All patients were admitted for intensive tar treatment of an exacerbation of their disease. This took the form of regularly increasing concentrations of either dithranol paste or crude coal tar applied to affected skin. None had ever received methotrexate therapy or had overt liver disease.

Disease extent was gauged by visual assessment, and blood samples were taken on admission, at 7 days, 14 days and weekly thereafter so long as tar therapy continued. All samples were taken at the same time of day for each patient.

Blood was withdrawn into plastic syringes; 10 ml was then placed in a plastic lithium heparin tube, all tubes having come from a single batch, whose zinc contamination had been assessed. This was acceptably low at 78±21 nmol/l. Needles and syringes used were not found to produce significant contamination of the blood samples during their brief contact.

Blood was kept chilled on ice until δ-ALA.D assay was performed, using the European Standardized Method (Berlin & Schaller, 1974), within 3 h of venepuncture.

The blood was then centrifuged, the plasma removed and stored at −4°C until zinc and copper analyses were performed. Copper was analysed because of its absorption linkage with zinc in man, alterations in one element thereby affecting the other. All analyses for each metal for each patient were carried out in one batch to minimize instrumental variation. Haemolysed specimens were discarded.

One millilitre of whole blood was retained for blood lead analysis.

Samples of the current topical application from each patient were similarly collected and assayed.

All trace metal analyses were performed on a Perkin Elmer 306 Atomic Absorption Spectrophotometer fitted with HGA 72 Gaphite Furnace. Blood lead was analysed at 283·3 nm, plasma zinc at 213·9 nm, plasma copper at 324·8 nm and ointment zinc at 307·6 nm.

## RESULTS

### *Diurnal variation*

The effect of diurnal variation on plasma zinc values was considered. Plasma zinc decreases during the day with an abrupt further decrease following each meal (Hughes, personal communication).

For this study blood was removed at a minimum of 2 h post-prandially, at either 9 a.m. or 2 p.m. The time of blood sampling was constant for each individual patient. It was found that the mean plasma zinc did not differ significantly between these two groups.

### *Ointment analyses*

When the elemental zinc content of the ointment was analysed and related to the surface area of involved skin in each patient, it was estimated that, over the 2-week study period, the mean application was 615 mmol zinc (41 mmol zinc per day).

This compares with an average whole body zinc content of 21·0–35·0 mmol, and a daily dietary zinc content of 0·23–0·3 mmol, absorption of which is variable (Davies, 1972). Thus even a small degree of systemic absorption of the application might be expected to have a demonstrable effect on zinc status.

### *Zinc analyses*

No significant increase in plasma zinc was seen in any individual over the 2-week study period. Three individuals, who had never before received tar therapy, were followed in more detail for a further three weeks, and again no increase was detected (Table 1).

TABLE 1. Plasma zinc (mean ± s.d. μmol/l) in psoriatic in-patients at Day 0 and Day 14 of hospital admission (normal range 14·4 ± 1·7 μmol/l)

| | Day 0 | Day 14 | '*t*'-test |
|---|---|---|---|
| All patients (n = 30) | 11·6 ± 1·8 | 11·5 ± 1·8 | NS |
| Extent < 10% (n = 9) | 11·9 ± 1·9 | 11·5 ± 2·1 | NS |
| Extent > 10% (n = 21) | 11·5 ± 1·7 | 11·5 ± 1·8 | NS |

It was observed, however, that the initial mean plasma zinc of the patient group was significantly lower than the mean plasma zinc of an age-matched control healthy population (n = 28, patients age 48·7 ± 12·7 years; control age 43·1 ± 14·3 years) when compared by Student's '*t*'-test, (patients zinc 11·6 ± 1·8 μmol/l; control zinc 14·4 ± 1·7 μmol/l, $P < 0·0001$). This is in agreement with the findings of Greaves & Boyde (1967), although Withers *et al.* (1968) found no such difference.

The patients were then split into two groups for analysis according to extent of disease and it was found that the plasma zinc of the more extensively affected group (> 10% of body surface area) was slightly lower at 11·5 ± 1·7 μmol/l (n = 21) than that of the milder group (< 10% of body surface area) at 11·9 ± 1·9 μmol/l (n = 9). This difference is not significant.

The aetiology of this disturbance of zinc distribution in psoriasis is uncertain, although several theories have been postulated (Molin & Wester, 1973).

### *δ-ALA.D analyses*

No significant change was found during the study period in this zinc dependent haem enzyme (Day 0 28·1 ± 9·4 nmol ALA/min/ml RBC; Day 14 30·1 ± 10·6 nmol ALA/min/ml RBC), and levels were not significantly different from those of the normal population (Meredith, personal communication), (patients δ-ALA.D 28·1 ± 9·4 nmol ALA/min/ml RBC; controls δ-ALA.D 31·8 ± 6·3 nmol ALA/min/ml RBC.

### *Lead analyses*

Blood lead levels were assayed in all patients to exclude excessive lead exposure, which might alter the above results. One patient was found to have an elevated blood lead and his results were discounted. The mean blood lead level of the remaining patient group was normal (1·0 ± 0·3 μmol/l).

### *Copper analyses*

Plasma copper was analysed on each occasion in all patients and showed no significant change over the study period. (Day 0 plasma copper 18·2±3·8 μmol/l; Day 14 plasma copper 18·1±3·7 μmol/l).

## DISCUSSION

Large quantities of zinc, as zinc oxide, are applied to abnormal skin in several chronic skin diseases. There is often increased local blood flow in these conditions and percutaneous absorption of certain substances may be high.

Cutaneous exposure to elemental zinc (mean 41 mmol per day, maximum 253 mmol per day in one patient) was very high in the psoriatic patients studied in relation to their normal exposure through diet (average 0·23–0·35 mmol per day).

Oral zinc supplementation of 600 mg zinc sulphate daily (3·7 μmol zinc) would provide a similar level of exposure and has been shown to exert a definite healing action in skin diseases associated with zinc depletion (Pories *et al.*, 1967; Hallbrook & Lanner, 1972). It is, therefore, possible that zinc absorbed from topical applications might exert a systemic effect. Hallmans (1978a) found significantly raised serum zinc levels in rats within 24 h of the application of topical zinc oxide to excisional wounds. He also (Hallmans, 1978b) observed a significant rise in serum zinc in eight acutely ill patients with major burns treated topically with zinc oxide tape. In this study, however, any such absorption was not reflected in increased plasma levels using a sensitive method of analysis (flameless atomic absorption spectrometry). However, plasma zinc does not clearly reflect the body's zinc stores and it is probable that zinc absorption did occur without a change in the plasma zinc level. Further work on this aspect remains to be done.

Blood δ-ALA-D also remained unaltered over the study period, as expected from the plasma zinc results.

The reduced plasma zinc levels found in our patients confirmed previous work (Greaves & Boyde, 1967). All patients were hospitalized for an exacerbation of their disease, but their plasma zinc levels remained low even after clinical improvement (plasma zinc 11·6±1·8 μmol/l on admission; 11·5±1·8 μmol/l after 2 weeks).

The clinical relevance of these abnormalities of zinc distribution to the course of psoriasis remains to be elucidated.

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## *Case Reports*

# Pemphigus vulgaris and pemphigus foliaceus coexisting with oral lichen planus

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## SUMMARY

Two women, aged 72 and 61 years, had mucocutaneous manifestations of pemphigus vulgaris, and one 76-year-old man had pemphigus foliaceus coexisting with oral lichen planus. Clinical and histopathological findings as well as direct and indirect immunofluorescence studies established the diagnosis.

The immunopathology of pemphigus is well established (Jordan *et al.*, 1974; Beutner *et al.*, 1975; Tuffanelli, 1975; Beutner, Jordan & Chorzelski, 1976; Dabelsteen, 1978) and several reports have described the coexistence of pemphigus with other diseases characterized by prominent immunological changes (Beutner *et al.*, 1976).

The epithelial changes of lichen planus (LP) lesions are invariably accompanied by an infiltrate of lymphocytes in the subepithelial zone. The presence of a cellular infiltrate similar to that associated with cell mediated hypersensitivity and the *in vitro* demonstration of cellular hypersensitivity to antigens extracted from oral LP lesions intimate an immunological aspect in the pathogenesis of LP (Holmstrup & Søborg, 1977).

Although coexistence of bullous pemphigoid and LP has been described there have been no previous reports on the coexistence of pemphigus and LP. The aim of this paper is to present three such patients from a group of 804 patients with oral LP, who since 1965 have been referred to and followed regularly at the Department of Oral Medicine and Oral Surgery, University Hospital (Rigshospitalet), Copenhagen.

## CASE REPORTS

### *Case* 1

On admission in July 1969 a 52-year-old woman presented with white asymptomatic lesions of the oral mucosa of unknown duration.

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Her previous medical history was non-contributory. She was in good health and was taking no medicines.

Clinical examination showed bilateral reticular white striae on the buccal mucosa and the inferior buccal groove. There were no skin lesions of LP.

The histopathology of a biopsy from the left buccal mucosa showed a typical picture of LP (Fig. 1).

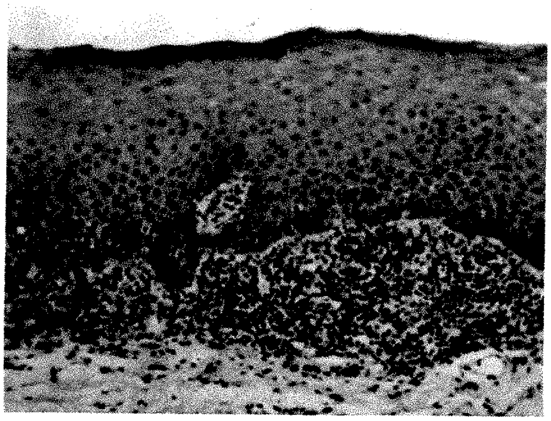

FIGURE 1. Case 1, July 1969. Microphotograph of oral biopsy exhibiting a normal differentiated, parakeratotic squamous epithelium with a band-like subepithelial lymphocyte infiltration compatible with the diagnosis of LP.

*Course and supplementary diagnosis.* The patient was seen once a year from 1969 to 1974. The LP lesions had gradually diminished, and as she had negligible subjective symptoms it was decided to terminate the regular follow-up.

However, in January 1978 the patient returned complaining of a 10-month history of 'stomatitis' and with a few solitary blisters on the skin. The blister spreading-test (Asboe-Hansen, 1960) was positive. The LP lesions of the oral mucosa were still present, and in the left buccal mucosa a ruptured bulla was observed.

The histopathology of the oral and cutaneous lesions exhibited suprabasal splitting with acantholytic cells, and the immunofluorescence (IF) findings were characteristic of pemphigus vulgaris (Beutner *et al.*, 1976; Dabelsteen, 1978) with intercellular deposits of IgG in all layers of the epithelium, and complement in the basal and parabasal layer (Fig. 2). Antiepithelial antibodies in serum (titre 1:8) were demonstrated.

*Case 2*

On admission in February 1965 a 59-year-old man had a 6-month history of painful sores on the tongue.

Since 1962 he had been suffering from sero-positive rheumatoid arthritis and since 1964 from psoriasis.

Clinical examination revealed an erosion on each side of the tongue with adjacent erythematous and whitish changes of the mucosa (Fig. 3). In the buccal mucosa bilaterally white reticular striae were seen.

The histopathology of a biopsy from the tongue was compatible with LP exhibiting a hyperorthokeratotic squamous epithelium with focal atrophy, saw-toothed rete pegs, thickening of the granular layer and a band-like subepithelial lymphocytic infiltrate.

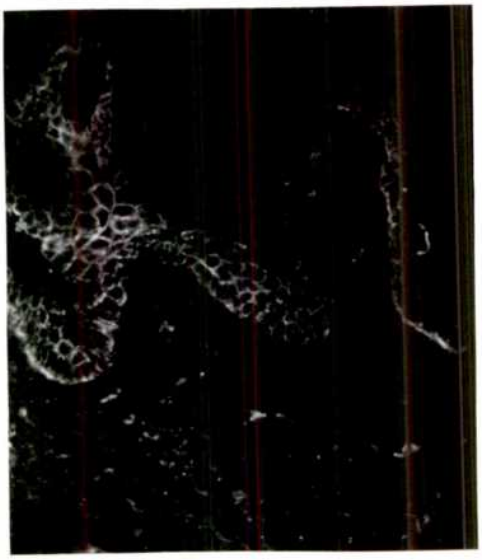

FIGURE 2. Case 1. Direct IF staining of tissue from the pemphigus vulgaris lesion in the left buccal mucosa, staining for complement. Positive staining is seen at the cell membranes in the basal and parabasal layers and in some areas of the basement membrane zone.

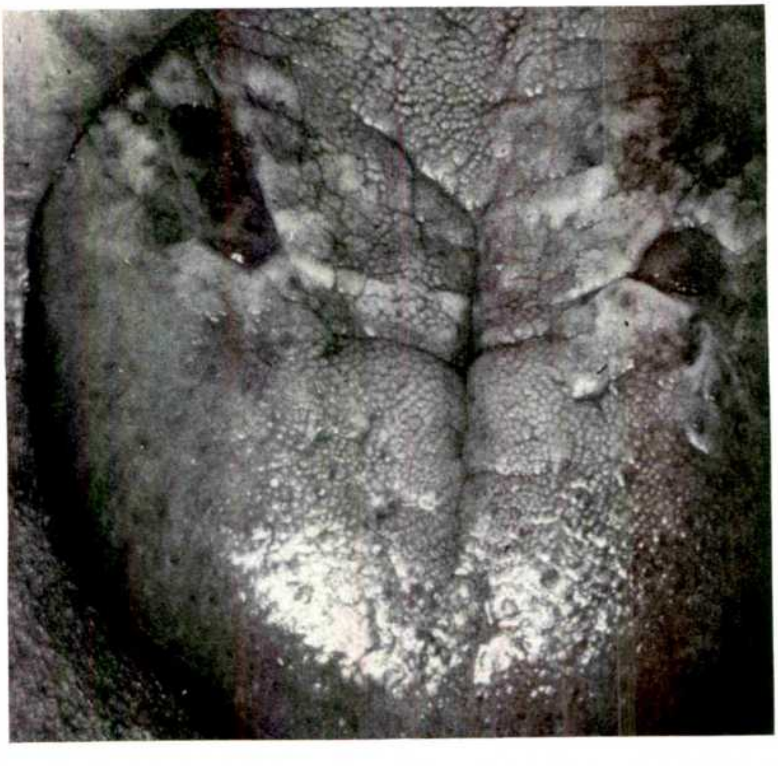

FIGURE 3. Case 2. February 1965. Note two fibrin-covered ulcers on the tongue with adjacent erythematous and whitish changes of the mucosa.

*Course and supplementary diagnosis*. Under the diagnosis of LP the patient was seen once a year from 1965 to 1978. The ulceration of the tongue healed, but the remaining lesions persisted.

In February 1978 he presented flaccid blisters, erosions and scaling crusted lesions on the skin, predominantly on the trunk and arms. The blister spreading-test and Nikolsky sign were positive. The buccal oral mucosa exhibited blisters and erosions.

The histopathology of a biopsy from the skin showed clefting and acantholysis in the granular layer as seen in pemphigus foliaceus. The IF demonstrated intercellular deposits of IgG and complement in the epithelium of the skin, and serum contained antiepithelial IgG (titre 1:20).

### *Case 3*

On admission in August 1976, a 72-year-old woman had a 4-month history of painful stomatitis with blisters of the oral mucosa and a general cutaneous eruption which had just developed.

Her previous medical history was non-contributory. She had been in good health and was taking no medicines.

On the trunk, abdomen, thighs, genitalia and in the axillae there were numerous itching erosions and a few bullae. The blister spreading-test was positive, while the Nikolsky sign was negative. Examination of the oral mucosa revealed pseudomembraneous ulceration with peripheral erythema of the lower vermillion border, the right and left buccal mucosa and the left margin of the tongue.

One year later the oral lesions had changed. Besides the previously described erosions, several parts of the mucosa showed white striae (Fig. 4). The histopathology was compatible with LP and

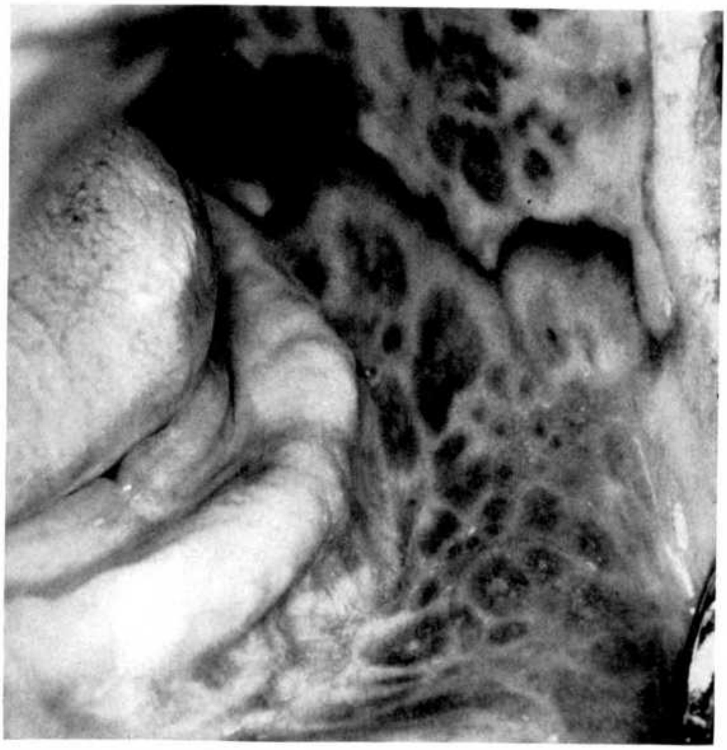

FIGURE 4. Case 3. Left buccal mucosa with fibrin covered ulceration and peripheral erythema. Below and in front of this area typical white reticular striae are seen on the labial mucosa.

showed an atrophic squamous epithelium with parakeratosis, liquefaction degeneration of the basal cell layer and saw-toothed rete pegs and a connective tissue with a cellular infiltrate composed mainly of lymphocytes. The IF findings of mucosa adjacent to the ulceration were typical of pemphigus vulgaris and showed intercellular deposits of IgG in all layers of the squamous epithelium and with complement in the basal and parabasal cell layer. The serum contained circulating anti-epithelial antibodies in a titre of 1:128.

## DISCUSSION

The coexistence of pemphigus with other diseases has been described in several reports (Beutner *et al.*, 1968; Chorzelski, Jablonska & Blaszczyk, 1968; Peck *et al.*, 1968; Bean & Lynch, 1970; Orfanos, Gartman & Mahrle, 1971; Stilmann & Baer, 1972; Chorzelski *et al.*, 1974; Naysmith & Hancock, 1976; Noguchi & Nishitani, 1976). LP has been reported with bullous pemphigoid (Stingl & Holubar, 1975; Sobel, Miller & Shatin, 1976; Saurat *et al.*, 1977). Our three cases seem to be the first reported cases of clinically, histopathologically and immunologically confirmed coexistence of LP and pemphigus.

The prevalence and incidence of LP and pemphigus in the Danish population are not known. A very rough annual incidence rate of 0·161 cases of pemphigus vulgaris per 100,000 was determined for the Jerusalem area Jewish population (over the age of 20), an area of known high prevalence (Pisanti *et al.*, 1974). If the same high incidence rate were valid for Denmark, one would expect about six new pemphigus vulgaris patients per year in the adult Danish population. A Swedish study of prevalence of oral LP, in a population comparable with the Danish population, showed a prevalence of 1·6% (Axéll, 1975). Making a hypothetical calculation using the binomial distribution (Therkelsen, 1974) on the above data, it could be shown that $P < 0{\cdot}004$ of meeting two or more of the expected six annual pemphigus vulgaris patients with coexisting LP.

Our findings may suggest the presence of a basic disturbance (immunological?) leading to a higher susceptibility for the two diseases (Benacerraf, 1974).

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# Amyloid vascular disease: cord-like thickening of mucocutaneous arteries, intermittent claudication and angina in a case with underlying myelomatosis

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## SUMMARY

A patient with established myelomatosis and an IgG paraproteinaemia presented with intermittent claudication. Indurated cord-like structures were noted on the dorsum of the right hand and the buccal surface of the lower lip, and biopsy of the hand lesion revealed massive amyloid deposition within the wall of an artery. Cord-like thickening of mucocutaneous arteries as a presenting sign of systemic amyloidosis in the absence of glossomegaly or other cutaneous lesions has not been reported previously. The presence of intermittent claudication and angina suggested that amyloid involvement of large arteries was extensively distributed.

## CASE REPORT

A 64-year-old man presented in 1968 with a 3-year history of slowly progressive intermittent claudication of the legs, and a 6-month history of claudication of the arms during shaving. Myelomatosis with an IgG paraproteinaemia had been diagnosed on the basis of an abnormal bone marrow in 1963, and he had been maintained on intermittent melphalan and systemic corticosteroids since then. A sero-negative arthritis had been first noted in 1953.

Examination of the cardiovascular system revealed bilateral absence of radial, ulnar, posterior tibial and dorsalis pedis pulses, and the peripheral arteries were thickened on palpation. A curious indurated cord-like structure was noted on the dorsum of the right hand in the interval between the thumb and index finger, and a similar tortuous cord was present submucosally on the buccal surface of the lower lip (Fig. 1). There was pseudohypertrophy of the calf and arm muscles, and deformity of the distal interphalangeal joints of several fingers. The remainder of the physical examination was normal; in particular, there were no other cutaneous lesions and glossomegaly was absent.

The following investigations were normal: haemoglobin, white cell count, platelet count, electrolytes and urea, serum creatinine, calcium, albumin and bilirubin. Osteolytic lesions were not seen on skeletal survey. The ESR was markedly elevated at 117 mm/h (Westergren). The sternal bone marrow contained 26% plasma cells with many immature forms. The alkaline phosphatase was raised at 60 K.A. units (normal <13) as was the SGOT at 33 iu/l (normal <25). The total serum protein was

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97 g/l (normal <74), the serum globulin was 56 g/l (normal <32) and serum protein electrophoresis showed a monoclonal band in the gamma region. Cryoglobulins and cold agglutinins were not detected. Serum immunoelectrophoresis revealed the presence of an IgG paraprotein. Serum IgG levels were very high at 54·5 g/l (normal range 7·0–14·5). Serum IgM concentration was normal at 0·45 g/l (normal range 0·4–2·3) but the serum IgA concentration was low at 0·45 g/l (normal range 1·0–5·0), indicative of an element of immune paresis.

Biopsy of the cord-like lesion on the right hand proved it to be a grossly thickened blood vessel. Bleeding was copious, and it seemed that the vessel had no capacity to retract. Biopsy of the lip lesion was therefore not attempted. Histology of the hand lesion showed an artery with marked thickening of the wall and diminution in the size of the lumen (Fig. 2). The intima was thickened and fibrosed, and the intimal elastic lamina was destroyed. The media was greatly thickened and replaced by dense hyaline eosinophilic material with the characteristic staining properties of amyloid.

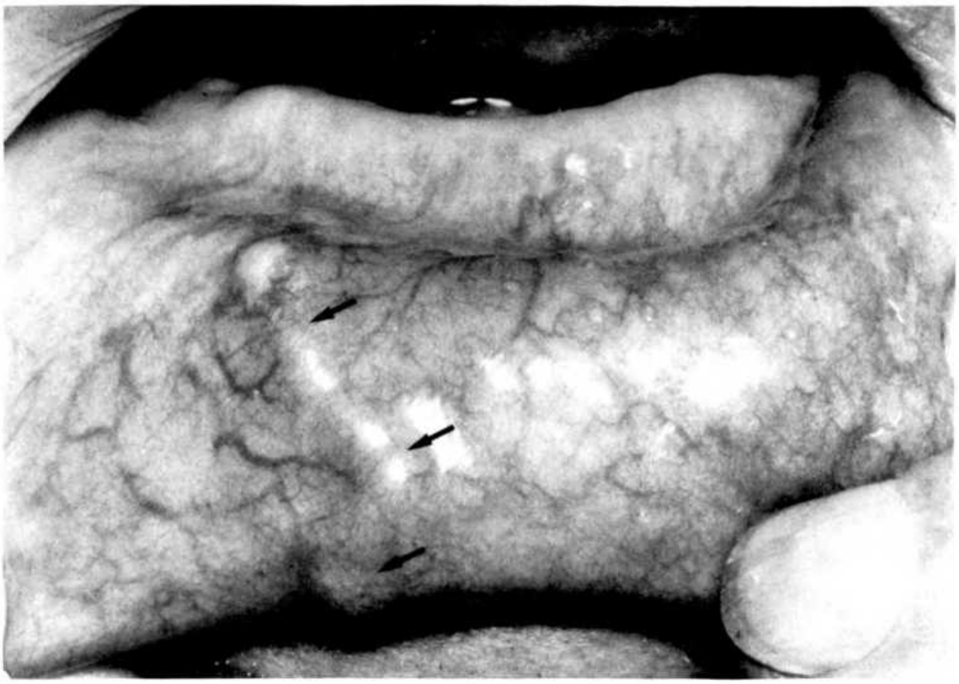

FIGURE 1. Tortuous cord-like thickening of an artery (arrowed) on the buccal surface of the lower lip.

The patient was treated by plasmapheresis in the hope that a reduction in the level of the abnormal circulating globulin might relieve the claudication, but his exercise tolerance improved only marginally. An acute phimosis with urinary retention secondary to venous thrombosis necessitated a dorsal split operation in October 1968. Angina pectoris developed in January 1969, and an ECG showed antero-lateral ischaemia. In June 1969 he suffered a right-sided central retinal artery thrombosis. Despite continued treatment with corticosteroids and intermittent melphalan and plasmapheresis, the serum IgG level remained very high and there was a gradual progression in the severity of the vascular symptoms, although haemopoietic and renal function were unimpaired. By January 1971 the patient was experiencing difficulty in finishing a meal due to aching in the arms and was reduced to climbing

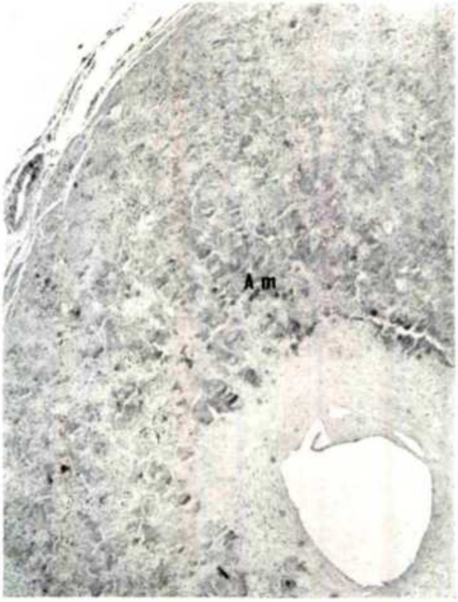

FIGURE 2. Segment of the wall of an artery showing massive amyloid infiltration. Am = amyloid (H & E, × 50).

the stairs on his hands and knees. He died in February 1971 of a myocardial infarction. An autopsy was unfortunately not performed.

## DISCUSSION

Amyloidosis develops as a complication of myelomatosis in about 15% of cases (Bayrd & Bennett, 1950). Cutaneous involvement is frequent in 'primary' and myeloma-associated amyloidosis and is often accompanied by glossomegaly. Cutaneous abnormalities in systemic amyloidosis have been extensively reviewed by Goltz (1952), Rukavina *et al.* (1956), Brownstein & Helwig (1970), Rubinow & Cohen (1978) and Breathnach & Black (1979). Purpuric and ecchymotic lesions on normal or clinically involved skin, and smooth, shiny, waxy amber-coloured papules, nodules and plaques occurring on the flexural areas are amongst the most characteristic lesions.

Amyloid infiltration of blood vessel walls, particularly the media of small arteries and arterioles, is widespread (Parkes Weber *et al.*, 1937; Eisen, 1946; Mathews, 1954; Symmers, 1956) and correlates with the clinical finding of purpura (Brownstein & Helwig, 1970). The massive infiltration with amyloid and resultant gross cord-like thickening of segments of mucocutaneous arteries seen in this patient, which occurred in the absence of glossomegaly or other cutaneous lesions, has not been described previously. Cooley (1953) reported rubbery nodulation along the course of the temporal arteries in a case of primary amyloidosis, but the lesions were not biopsied.

This patient showed evidence of extensive vascular disease, with symptoms of peripheral arterial and coronary insufficiency, and episodes of thrombosis. Postural hypotension (Gaan *et al.*, 1972), thromboembolism (Catovsky *et al.*, 1970; Barclay, Cameron & Loughridge, 1960) and angina and myocardial infarction (Brandt, Cathcart & Cohen, 1968; Barth *et al.*, 1970; Buja, Khoi & Roberts, 1970) are recognized complications of systemic amyloidosis. Intermittent claudication in association with amyloidosis has only rarely been reported. Parkes Weber *et al.* (1937), and Barnard, Smith &

Woodhouse (1938) each described one case, and Bero (1957) recorded two cases with arterial occlusion which in one case led to ischaemic necrosis of a limb. Zelis, Mason & Barth (1969) reported a further four cases with severe amyloid infiltration of the walls of muscle arterioles, and suggested on the basis of plethysmograph studies that claudication might be at least in part due to inability of diseased arterioles to dilate during exertion, mimicking the 'stiff heart' functional defect in amyloid heart disease (Chew *et al.*, 1975). Vessel wall rigidity with loss of retractile capacity was probably the main cause of the prolonged haemorrhage during biopsy observed in this case. Maldonado *et al.* (1973) were able to relieve claudication by plasmapheresis in a case of amyloidosis with cryoglobulinaemia. Plasmapheresis was unsuccessful in relieving symptoms in this case, however, suggesting that the hyperglobulinaemia was not responsible for the claudication. Considering the extent of histologically proven amyloid infiltration of blood vessel walls present in 1968, it is remarkable that this patient survived for a further 3 years before succumbing to a fatal episode of coronary vascular occlusion.

## ACKNOWLEDGMENT

We are grateful to Professor G.Wetherley-Mein for permission to report this case.

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# Genetic tylosis with malignancy: a study of a South Indian pedigree

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## SUMMARY

Tylosis at birth was found as an autosomal dominant inheritance in twenty-two members of a family spanning over five generations. Of those affected three of the fourth generation tylotics developed squamous carcinoma of the tylotic skin and one of these died of carcinoma of the oesophagus. It is possible that there is a genetic association between the state of the oesophagus and the tylosis of the palms and soles which predisposes both sites towards the development of squamous carcinoma.

Howel-Evans *et al.*, (1958) reported the association of tylosis with carcinoma of the oesophagus. In their study of forty-eight members of two families in Liverpool, who had developed tylosis of the late onset type, seventeen developed squamous carcinoma of the oesophagus. Shine & Allison (1966) reported carcinoma of the oesophagus in a tylotic in his sixty-first year of life. He had dysphagia from childhood but the tylosis became noticeable only during adolescence. Thambiah *et al.*, (1975) reported a 26-year old male who developed tylosis after a surgical by-pass was carried out for post-corrosive stricture of the oesophagus. Sequeira & Ingram (1947), as quoted by Shine & Allison (1966), reported seeing a tylotic patient in whom an epithelioma arose from the hand but no pedigree or case report was available.

## CASE REPORT

The propositus, his affected mother and affected elder brother were questioned separately regarding the family history. The requested information was age, sex, presence or absence of the tylosis, occurrence of growths and history of dysphagia in the affected individuals. The cause of death in the deceased tylotics, if known, was enquired into. Since the condition was a striking one present from birth, historical evidence of presence or absence of tylosis can be considered to be reliable. The growths on the skin of IV–1 and IV–11 were found to be squamous carcinoma from the medical records of the patients. The oesophageal growth of IV–1 was a squamous carcinoma.

The results are summarized in the pedigree chart shown in Fig. 1. Twenty-two members of this family spanning five generations were found to have tylosis at birth.

Patient IV–1 developed an ulcer on the right heel when he was 29 years old. Biopsy revealed this to

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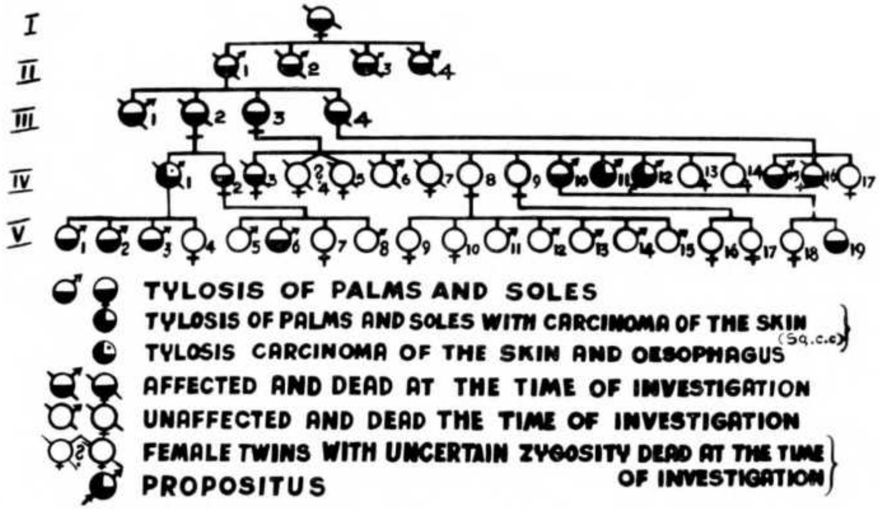
I
II
III
IV
V
TYLOSIS OF PALMS AND SOLES
TYLOSIS OF PALMS AND SOLES WITH CARCINOMA OF THE SKIN (Sq.c.c)
TYLOSIS CARCINOMA OF THE SKIN AND OESOPHAGUS
AFFECTED AND DEAD AT THE TIME OF INVESTIGATION
UNAFFECTED AND DEAD THE TIME OF INVESTIGATION
FEMALE TWINS WITH UNCERTAIN ZYGOSITY DEAD AT THE TIME OF INVESTIGATION
PROPOSITUS

FIGURE 1. Pedigree chart slowing autosomal dominant inheritance. Figures represent the numbers of siblings. Twenty-two out of forty-five persons affected.

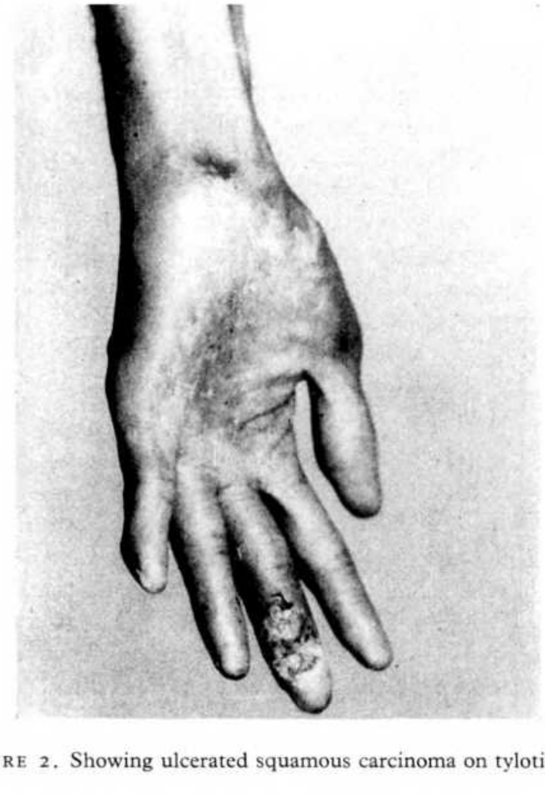

FIGURE 2. Showing ulcerated squamous carcinoma on tylotic skin.

be a squamous carcinoma and it was excised and skin grafted. He had no further complaints until he reached the age of 40 years when he developed dysphagia which was found to be due to a squamous carcinoma of the lower end of the oesophagus. The patient refused surgery and in a few months the obstruction became total and necessiated gastrostomy. A few weeks later he died. Autopsy was not done.

Patient IV–11 developed an ulcer on the plantar surface of the right big toe when he was 29 years old. Since it was a squamous carcinoma it was excised and skin grafted. The graft did not take and eventually the toe had to be amputated. He is now 37 years old and is quite healthy and has no dysphagia.

The propositus, who is 28 years old, developed an ulcer on the tylotic skin of the left middle finger 4 years ago. The ulcer slowly increased in size (Fig. 2). Biopsy showed squamous carcinoma. The ulcer was excised and skin grafted. A few of his finger nails showed pterygium formation.

The mother of the propositus III–3 also has tylosis from birth but no growths have developed and she has no dysphagia.

In none of the above three cases was the tylotic skin fissured nor was there any hyperhidrosis; on the whole the condition was asymptomatic.

The other tylotics on historical evidence did not have growths on the skin or dysphagia and in those who have died death was due to some unrelated condition.

## DISCUSSION

The tylosis occurred at birth in all the affected individuals in this pedigree unlike in other reports in the literature of tylosis associated with carcinoma of the oesophagus, where it was of the late onset type. The mode of inheritance in our patients appears to be autosomal dominant.

In the three patients who developed carcinoma on the tylotic skin there was no history of arsenical intake or of exposures to any known carcinogens, occupational or environmental. All of them developed the skin cancer in the second decade of life, a rather early age for squamous carcinoma to occur. Hence it is probable that the malignancy in these cases occurred due to genetic predisposition. To our knowledge only one other case report (Saqueira & Ingram, 1947) of squamous carcinoma arising on genetic tylosis has been reported in the literature. The chronological sequence of events in the tylotic who died of cancer (IV–1) was tylosis at birth, skin cancer in the second decade and cancer of the oesophagus in the fourth decade. It is therefore mandatory to follow the other two surviving tylotics with skin cancer and also the fifth generation tylotics to see if they develop further growths.

It was postulated by Allison & Shine (1966) that the combination of tylosis and oesophageal abnormalities is determined by a single autosomal gene. In our cases also it is possible that a single autosomal gene is responsible for the tylosis, the squamous carcinoma of the skin and the carcinoma of the oesophagus. Or the association may be polygenic. It has been established that there is a polygenic basis for neoplasia in certain strains of inbred mice (Moynahan, 1973) and the appearance of tumours depends on the presence of several genes in specific allelic form, although each gene alone is harmless.

In the light of this study, genetic tylosis should be considered not only as a skin marker of oesophageal carcinoma but also as a precancerous dermatosis.

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# Diffuse cutaneous mastocytosis with multiple cutaneous mastocytomas

## REPORT OF A CASE WITH CLINICAL, HISTOPATHOLOGICAL AND ULTRASTRUCTURAL ASPECTS

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## SUMMARY

A patient with diffuse cutaneous mastocytosis, present since birth, developed several cutaneous mastocytomas without any symptoms after puberty. Although the histological picture of the tumours, showing nuclear atypia, mitotic figures and varying metachromasia of the granules, raises suspicion of systemic involvement, this has not appeared so far.

The absence of symptoms could be due to the synthesis of an abnormal secretory product by the mast cells, which is supported by the absence of oedema and dilated blood vessels in the presence of extensive degranulation and by the unusual ultrastructural picture of the mast cell granules, i.e. little electron density with the almost complete absence of lamellar and crystalline structures.

It was not until 1951, more than 80 years after Nettleship's (1869) first description of the cutaneous manifestation of mastocytosis, that Degos *et al.* (1951) published the first report on diffuse cutaneous mastocytosis, a distinctive form of the mastocytosis syndrome with the following characteristics: varying degrees of erythema with a yellowish-brown pigmentation, sparing the palms and soles, and sometimes also the face; peau d'orange appearance of the skin; increased folds in the axillary and inguinal regions; nearly always severe pruritus and urtication; blistering is quite common.

The disease usually has its onset in early infancy, although onset in adult life has also been described (Degos *et al.*, 1951, 1952). The clinical course is very variable and is determined by the age of onset of the disorder. When the onset is in early life, most symptoms have a tendency to decrease or even disappear with age. Spontaneous regression of the skin lesions has never been documented in those rare cases in which diffuse cutaneous mastocytosis developed after puberty.

Systemic involvement may occur, especially in liver, spleen, lymph nodes and bones. Gastro-

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intestinal disturbances, haemorrhagic tendencies with or without abnormalities in the coagulation mechanism and episodes of flushing have also been reported (Degos *et al.*, 1951; Warin & Hughes, 1963; Burgoon, Graham & McCaffree, 1968; Woerdeman, 1970). According to Burgoon *et al.* (1968) diffuse skin involvement is rarely present without systemic involvement, although skin changes can be present for many years before the visceral expression of systemic mast cell disease becomes apparent. On the contrary, Haensch & Ippen (1968), describing diffuse cutaneous mastocytosis on the basis of two patients of their own and twenty cases from the literature, stated that this type is always limited to the skin.

Systemic involvement does not imply a bad prognosis, because spontaneous regression of visceral lesions is likely (Robinson *et al.*, 1962; Woerdeman, 1970). However, a case with a fatal outcome due to progression into a malignant mast cell leukaemia has been described by Waters & Lacson (1957). In early infancy a fatal outcome is also possible as a result of shock, caused by severe diarrhoea, vomiting and dehydration, due to the release of excessive amounts of histamine from the mast cells (Robinson *et al.*, 1962).

The present report is of a case of diffuse cutaneous mastocytosis in which large cutaneous tumours developed after puberty, while the patient has no symptoms.

## CASE REPORT

The patient is a 27-year-old male, who was seen as a 2½-year-old boy at our department, for the first time in 1954, for a generalized erythematous dermatosis which had been present from birth. At that time there was a brownish-red discolouration all over the skin, sparing only the palms and soles. Exaggeration of the axillary and inguinal skin folds, as well as accentuation of the normal skin markings were noted (Fig. 1a). On stroking the skin slight urtication sometimes occurred. Except for occasional mild pruritus there were no complaints. There was no history of blistering. The family history was non-contributory. General examination was normal and the blood count was within normal limits. X-rays of the thorax, pelvis and long bones showed no osseous involvement. In a skin biopsy an increased number of mast cells was found in the corium. The diagnosis 'urticaria pigmentosa congenita fere diffusa' was made on both clinical and histological criteria.

In 1978 the patient attended our department again, not because of any complaint, but for genetic counselling. The diffuse cutaneous involvement was unchanged, but for approximately 5 years large cutaneous tumours had appeared, initially on the lower legs and later also on the arms and thighs. Some of them had already disappeared spontaneously. He had no complaints, especially no pruritus and urtication, not even after stroking or scratching, no flushing, no gastro-intestinal disturbances and no haemorrhagic tendencies. The patient enjoyed excellent general health.

The skin still showed a generalized erythroderma, sparing only the palms and soles. The colour ranged from purple-red to yellowish-brown. However, the neck, parts of the antecubital and popliteal fossae, parts of the sacral region and buttocks and the pubic and lower abdominal regions were far less pigmented and had a more spotty appearance. Dozens of red or livid papules and nodules, scattered over the shoulders, chest, upper arms and upper legs, were superimposed on the diffuse erythematous skin changes. On the lateral part of both thighs an extensive infiltration was palpable. Neither exaggeration of the skin folds nor accentuation of the normal skin markings were seen any more (Fig. 1b).

The most remarkable finding was the presence of large cutaneous tumours with a diameter from 2 to 4 cm on both arms and legs. On the lower legs they had become confluent (Fig. 1c). These tumours showed sharply demarcated borders and a lobulated surface, and they were firmly attached to skin and

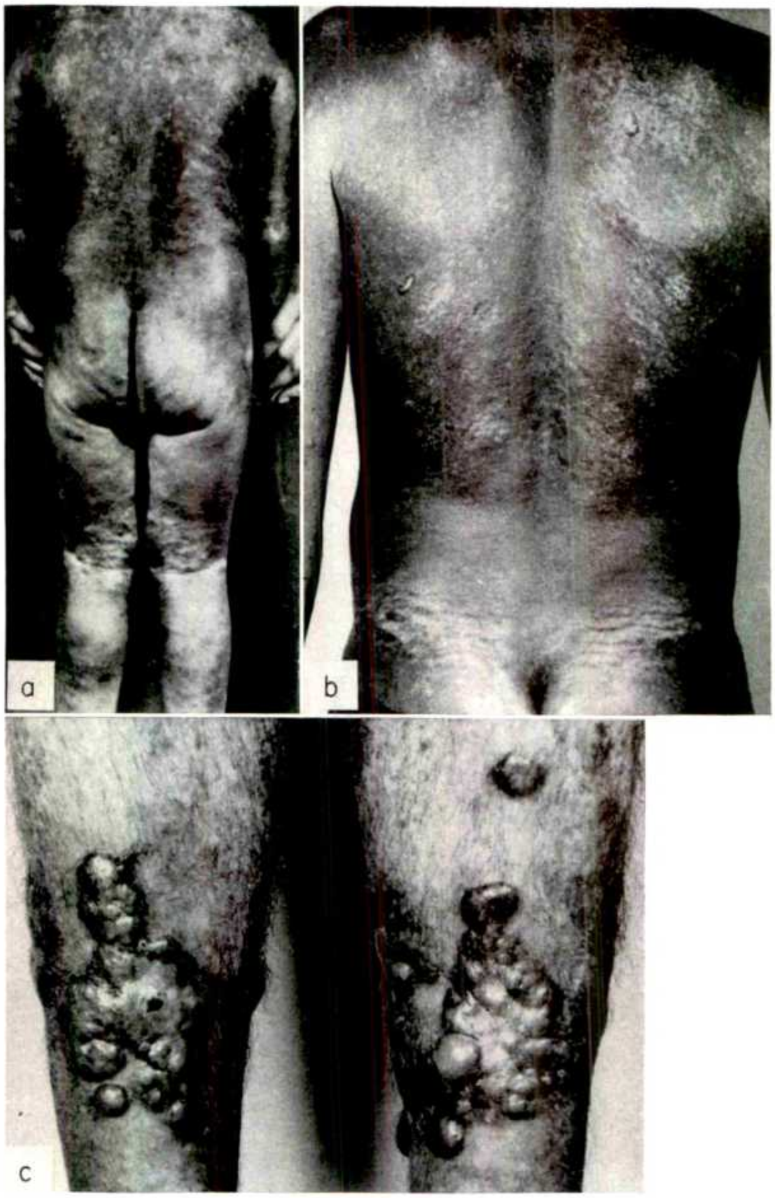

FIGURE 1. (a) Back of the patient in 1954, showing diffuse cutaneous involvement with exaggeration of the skin folds. (b) Back of the patient in 1979, still showing a diffuse erythema. The peau d'orange appearance and the exaggeration of the skin folds have disappeared. (c) Cutaneous tumours on both lower legs.

underlying structures. On both areolae of the breasts plaque-like lesions were found. The general examination was essentially normal; there were no enlarged lymph nodes, liver or spleen.

### *Laboratory investigations*

Haemoglobin, total and differential white cell count, sedimentation rate, liver function tests, serum creatinine, urea and electrolytes were all within normal limits. Platelet count, bleeding, clotting and prothrombin times were all normal. Urine content of histamine was 36 μg/24 h (normal value: 196 ± 116 μg/24h).

A toluidine blue-stained thick smear from the peripheral blood did not show mast cells. X-ray examination of chest, skull, pelvis and long bones did not reveal osseous involvement. Sternal bone marrow biopsy was not performed.

### *Histopathology*

The haematoxylin-eosin and toluidine blue stained sections from a maculopapular lesion showed loosely arranged spindle shaped mast cells, localized perivascularly throughout the dermis. Furthermore, some eosinophil granulocytes and melanophages were seen. In the lower dermis an increase of collagen fibres was present. The biopsy taken in 1954, also from a maculopapular lesion, showed the same histological picture.

The biopsy taken from the tumour on the right lower leg showed a dense extensive dermal infiltrate, consisting largely of mast cells, intermingled with several eosinophil granulocytes and lymphocytes, some of which had a cerebriform nucleus. The nuclei of many mast cells showed atypia to a varying degree, the nuclei being moderately polymorphous (Fig. 2a and b). Several mitotic figures were found in mast cells (Fig. 2a). Among the mast cell masses there were many small blood vessels with swollen

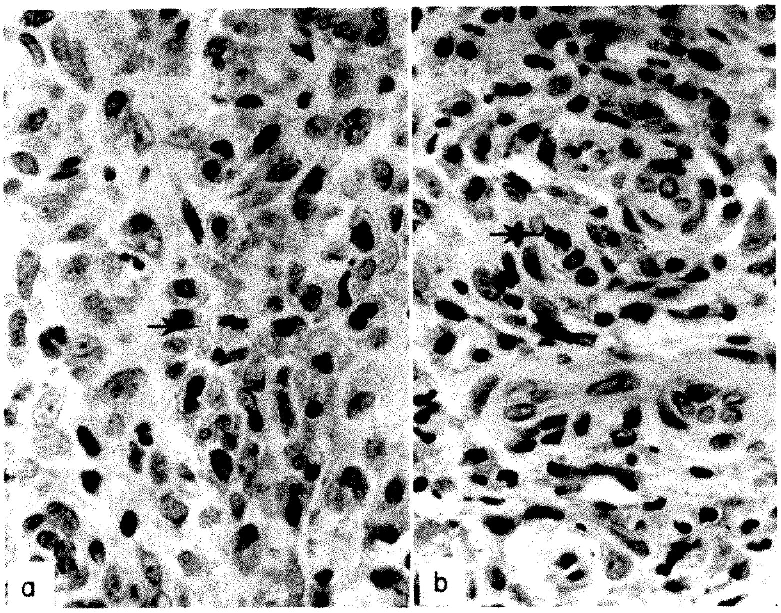

FIGURE 2. Micrograph of the dense mast cell infiltrate, showing the moderate nuclear polymorphism and the mitotic figures (↗) of a mast cell (a) and of an endothelial cell (b). Note the numerous blood vessels with swollen endothelium (H & E and toluidine blue, × 320).

endothelium. A few mitotic figures within endothelium were found (Fig. 2b). There was no appreciable oedema and no telangiectasia.

*Transmission electron microscopy (TEM)*

From a tumour on the right lower leg a biopsy was taken, minced into small pieces, immediately fixed in 1·5% sodium cacodylate buffered gluteraldehyde, and routinely processed for TEM. From three blocks ultrathin sections were made and after staining examined with a Philips 201 EM. A spectrum from typical to moderately atypical cells could be distinguished. The typical mast cells were often slender and fusiform and showed the following characteristics: several long and fine cytoplasmic processes, many rounded granules with a diameter varying from 150 to 750 mm, a not prominent endoplasmic reticulum and Golgi apparatus, a finely fibrillar cytoplasm, several mitochondria and some free ribosomes.

The granules showed a varying electron density, but were mostly less electron dense than those in the adjacent eosinophil granulocytes. An incomplete membrane surrounded some of the granules, and was locally fused with the cell membrane. Locally, several empty vacuoles were found. Many granules were disintegrated and showed a fine granular appearance with a compact core and peripherally a translucent zone. Only remnants of lamellar structures and no crystalline structures were found. Outside the cells only a few disintegrated granules were seen. Occasionally in cell processes a granule was present. The nuclei were oval with an irregular contour, a moderately broad rim of heterochromatin, a finely granular compact euchromatin, and a relatively small nucleolus. Several rather large atypical mast cells were seen. These possessed fewer cell processes, tended to have fewer granules, and

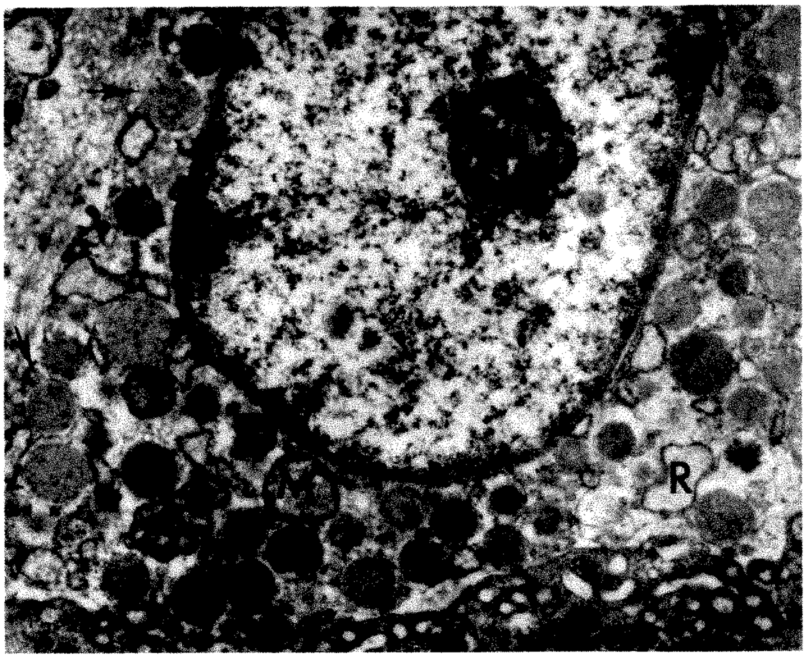

FIGURE 3. Electron micrograph of a part of an atypical mast cell with varying electrondensity of the granules, degranulation of the vacuolization-expulsion type (↗), dilated rough endoplasmic reticulum (R), little euchromatin and a conspicuous nucleolus (N). The granules show a finely granular structure, devoid of lamellar and crystalline structures. M = mitochondrion (uranyl acetate and lead citrate, × 12,000).

showed a prominent smooth and rough endoplasmic reticulum with dilated cisterns and a finely fibrillar matrix. The nuclei were plump, somewhat irregular with little heterochromatin, finely granular, loosely packed euchromatin and a conspicuous nucleolus (Fig. 3). Furthermore, immature mesenchymal cells resembling fibroblasts were encountered, frequently lying close to mast cells.

Locally, Charcot–Leyden crystals were found, mostly in the vicinity of eosonophil granulocytes.

## DISCUSSION

In our patient all the characteristic skin changes of diffuse cutaneous mastocytosis were present, although the very mild pruritus and urtication, and the absence of blisters are uncommon. The most remarkable finding is the presence of large cutaneous tumours, which appeared in his early twenties. To the best of our knowledge these have never before been described in diffuse cutaneous mastocytosis. However, in a patient with a congenital maculopapular form of mastocytosis Bakos, Storck & Silveiro Netto (1972) described the development of similar large cutaneous tumours on the arms, legs and trunk. Just as in our patient these tumours appeared after puberty. The appearance of these tumours was associated with episodic flushing, weakness, gastro-intestinal symptoms, dyspnoea and episodes of profuse bleeding from the tumours after local trauma. General examination showed weight loss, slight ascites, hepatosplenomegaly, generalized lymphadenopathy and muscle wasting. In a 2½-year-old boy with the nodular type of cutaneous mastocytosis Kierland & Stegmaier (1950) described elevated plaques and tumours, varying in diameter from 1 to 4 cm, scattered over the trunk, neck and limbs. In the literature no other cases with similar multiple tumours were found.

Returning to our patient, the question arises whether we are facing a disease still limited to the skin, or whether the appearance of more and more tumours must be regarded as a sign of incipient internal dissemination, as in the patient described by Bakos *et al.* (1972). Extensive physical and laboratory investigations did not reveal any indication of systemic mast cell disease. However, in contrast to the monotonous cytological appearance of nodular cutaneous mastocytosis and the solitary mastocytoma as described by Mihm *et al.* (1973), in our case nuclear polymorphism with conspicuous nucleoli and mitotic figures in mast cells were found, raising suspicion of systemic involvement (malignant reticulosis; Lennert, 1962; Lennert & Parwaresch, 1979).

Theoretically, the absence of symptoms and signs after stroking the skin could be due either to failure of mast cell degranulation or to the synthesis of a functionally abnormal secretory product by the mast cells. In the ultra-thin sections many mast cells showed evidence of degranulation, mostly of the vacuolization–expulsion type (Moriyasu & Yamura, 1973). The possibility of the synthesis of an abnormal secretory product is supported by the absence of oedema and dilated blood vessels in the presence of extensive degranulation, and by the absence of elevated urinary histamine excretion, in contrast to Demis' finding of elevated histamine levels in 24 h urine collections of patients with mastocytosis (Demis, Walton & Higdon, 1961). Furthermore, the ultrastructure of the granules, i.e. unusually little electron density with lamellar and crystalline structures practically lacking, could be associated with abnormal function. Since extensive physical and laboratory investigation did not reveal any indication of systemic mast cell disease, we do not intend to give the patient any treatment, although regular examination will be necessary.

The familial occurrence of (cutaneous) mastocytosis has been reviewed by Shaw (1968) and Bazex *et al.* (1971). Because there is no consensus as to the mode of transmission and the degree of penetrance, a reliable estimate of the risk for his offspring is not possible.

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## *Brief Communication*

# Immunohistology and autoantibody studies in alopecia areata

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We should like to place on record the results of an investigation carried out in 1975, because although the findings of a number of other investigations of immunological aspects of alopecia areata have been published (see Klaber & Munro, 1978), our findings appear still to be of interest.

## MATERIALS AND METHODS

Large scalp biopsies were taken from bald areas from ten patients with alopecia areata, self-selected primarily by their enthusiasm to cooperate. Four had a personal history of atopic disorders, two had 'autoimmune' disorders, three gave a history of atopic disorders in first-degree relations and one gave a history of autoimmune disease in a first-degree relation.

The biopsies were submitted to routine histological examination and to direct immunofluorescence for *in vivo* deposits of IgG, IgM, IgD, IgE, complement, albumin and fibrinogen. Monospecific horse antisera to these components were obtained from the Netherlands Red Cross and conjugated in our laboratory with fluorescein isothiocyanate (FITC). The unabsorbed conjugates had a fluorescein to protein molar ratio (F:P) of approximately 4 and were purified by gel filtration through Sephadex G-25 (Pharmacia) and absorbed with homogenates of rat liver, kidney and gastrointestinal tract and human skin so that they gave no staining reaction on normal human skin. The absorbed conjugates were used at a protein concentration of 0·8 g/100 ml.

The sera of nine of the patients were examined by routine indirect immunofluorescence techniques (Nairn, 1976) for autoantibodies: antinuclear factor, antigastric parietal cell, antismooth muscle, antimitochondria, antithyroid cytoplasm and colloid, antireticulin and antiskin (epidermal components and follicles; substrates human, rat, rabbit, mouse, and guinea-pig skin). The conjugate used was a FITC-conjugated goat antihuman gamma globulin (F:P = 4·7; protein concentration 0·9 g/100 ml).

After immunofluorescence staining, the microscopic preparations were examined by dark-ground UV fluorescent microscopy using a condenser fitted with a toric lens beneath and a colourless barrier filter.

In addition all sera were tested for antithyroglobulin with tanned sheep red cells sensitized with thyroglobulin (Burroughs Wellcome) and for rheumatoid factor with latex particles coated with human globulin (RA test, Hyland Laboratories). IgE levels were estimated by radioimmunoassay (Phadebas Kit, Pharmacia).

## RESULTS

The scalp biopsies showed the well-known histology of alopecia areata and there were no obvious differences between the ten biopsies. Direct immunofluorescence examination of biopsies was uniformly negative; no immunoglobulin, complement or fibrin were detected. Gastric parietal cell antibodies were present in one patient, and rheumatoid factor in another, but no other autoantibodies

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in any of the nine sera examined. In particular, no antibodies were detected to epidermal cells or hair follicles. IgE levels were normal in seven, but elevated (1480 ng/ml and 2550 ng/ml) in two.

## DISCUSSION

Unlike other autoimmune conditions no antibodies to epidermal elements and hair follicles were found in the present study either in the patients' sera or bound *in vivo* in the scalp biopsies. The latter finding agrees with the recent report of Klaber & Munro (1978).

Likewise no increase in the incidence of thyroid or other autoantibodies was observed, confirming the earlier observations of Cunliffe *et al.* (1969). Most biopsies showed prominent lymphocytes and histiocytes around the hair bulb and/or invading the follicle; blast cells were not observed. The immunohistology suggests a T-cell mediated immune reaction rather than antibody induced tissue damage. However, whether this is directed against the hair follicle, and is a primary or secondary event, is yet to be determined.

The two patients with raised IgE levels were both atopic subjects but our findings were otherwise similar in patients with atopic disorders, with autoimmune disorders or with neither.

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## *Comment*

# PHOTO-CARCINOGENICITY: THE PHYSICAL BASIS OF ITS EXOGENOUS CAUSES

Phototherapy—in contrast to methods of photochemotherapy—is gaining favour in the treatment of dermatoses owing to the absence of a need for combining radiation with medication. For practical purposes the range of wavelengths 240–320 nm is generally considered carcinogenic. The quantum energy $E = hf$ at wavelength 320 nm (frequency $f = 937{\cdot}5 \times 10^{12}\ s^{-1}$ and Planck's Universal Constant ($h = 6{\cdot}626 \times 10^{-34} Js^{-1}$) is $6{\cdot}2 \times 10^{-19}$ J or, as a round figure, $E = 6 \times 10^{-19}$ J. This, then, is the lowest energy of radiation capable of producing a skin cancer. Ultraviolet rays of wavelength 320 nm penetrate into the epidermis sufficiently deep to reach the stratum basale, and it is there that they are absorbed. Wavelengths shorter than 320 nm carry greater quantum energies E, owing to the fact that wavelength and frequency are inversely proportional. To assess the risk of photo-carcinogenicity three different sources of ultraviolet radiation are considered; namely, natural sunshine, therapeutic irradiation, and cosmetic radiation, in this order.

## SOLAR RADIATION

Natural sunshine reaches the terrestrial atmosphere within the entire range of its extraterrestrial (cosmic) spectrum. The chemical composition of the air causes it to act as a complex optical filter, absorbing all rays with wavelengths shorter than 292·5 nm. About 30 miles (50 km) above the earth the short ultraviolet rays (below 220 nm) of solar origin disrupt the oxygen molecules $O_2$ producing $O_3$ (ozone) according to the overall formula $3\ O_2 + hf \rightarrow 2\ O_3$. Ozone absorbs all rays shorter than 292·5 nm thus creating a protective mantle enveloping the entire earth and enabling life of the terrestrial variety to develop and to exist. While the short ultraviolet rays reached the earth in bygone periods of evolution, life of the kind we know could not exist because the short rays, being highly inimical to life, are absorbed by the protein of bacteria, etc. and would have inhibited any evolutionary developments.

The optical filtering effect of today's terrestrial atmosphere permits rays between 292·5 and 800 nm to reach the surface of the earth with relatively little loss of energy in the atmosphere. The 'orange' and 'red' light rays penetrate without deviation from their extraterrestrial direction of propagation, but the short 'blue' and 'violet' rays of luminous wavelengths are severely scattered and reflected while traversing the atmosphere. This applies to a far greater degree to the still shorter ultraviolet rays. Normally, it is only the molecules of the atmospheric gas mixture (mostly oxygen and nitrogen) which scatter the ultraviolet rays but during the last 40 years or so industrial pollution (smoke, soot, dust, etc.) has greatly aggravated the losses of solar energy owing to increased scattering and reflection. The lower limit of atmospheric transparency is still taken as at 292·5 nm of solar radiation, but that is merely a theoretical value which is realized only at high altitudes in mountainous country well away from human occupation and air pollution. This is clearly demonstrated in Table 1 which quotes solar intensities obtained at different sea-levels (Bener, 1972).

The lower limit of atmospheric transparency rises with increasing aerial pollution. It follows that people living in industrial areas are better protected against solar damage to their skin than people living in the open country.

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TABLE 1. Solar intensities at different altitudes with the sun in its zenith (solar height h = 90°)

| Altitude | Solar intensity ($W m^{-2}$) |
|---|---|
| High mountains | 1190 |
| Plains | 1130 |
| Large cities | 1070 |
| Industrial areas | 1000 |

With the sun nearing the horizon the aerial path of solar rays increases by a factor $m = \sin^{-1} h = \operatorname{cosec} h$ where h is the height of the sun above the horizon and m is the aerial mass. With the sun in its zenith (h = 90°) the factor m = 1. With the setting sun only 5° above the horizon, m = 11·5 Consequently, the values quoted in Table 1 are greatly reduced (Table 2). Of these global intensities only fractions pertain to the ultraviolet spectrum. The values given in Table 3 show clearly that the shorter wavelengths (UV-B) suffer greater losses while traversing the atmosphere than do the longer wavelengths (UV-A).

The ranges of ultraviolet radiation are divided into UV-C, UV-B and UV-A. The spectral ranges are as follows:

UV-B: 280–315 nm (Forbes, 1972) or 280–320 nm (Magnus, 1972) these values being used generally in medical texts.
UV-A: 315–380 nm or 320–380 nm the longwave end sometimes, but wrongly, being extended to 400 nm. The range 380–400 nm already forms part of the luminous (visible) spectrum.

The solar constant is, as the name implies, a characteristic datum of solar radiation; it is the amount of radiation striking a surface of 1 $m^2$ perpendicularly to the direction of the incident rays during 1 min. Table 4 shows values of the solar constant which apply to the average distance of the sun from the earth. Owing to the elliptical orbit of the earth the average distance ($149·6 \times 10^6$ km) decreases in January to $147·1 \times 10^6$ km (perihel) and increases in July to $152·1 \times 10^6$ km (aphel) thus alternating the extraterrestrial constant from 1360 $W m^{-2}$ to 1410 $W m^{-2}$ in winter and to 1310 $W m^{-2}$ in summer. Terrestrial values fluctuate accordingly between 1128 $W m^{-2}$ and 987 $W m^{-2}$ in winter (at 3500 m and at sea-level), and between 1048 $W m^{-2}$ and 917 $W m^{-2}$ in summer (at 3500 m altitude and at sea-level). The solar constant is usually dimensioned $mW cm^{-2}$ but is here quoted as $W m^{-2}$

TABLE 2. Solar intensities at different altitudes with the sun near the horizon (solar height h = 5°)

| Altitude | Solar intensity ($W m^{-2}$) |
|---|---|
| High mountains | 70 |
| Plains | 50 |
| Large cities | 40 |
| Industrial areas | 30 |

TABLE 3. Average solar radiation intensities on the surface of the earth

| | Solar intensity (W $m^{-2}$) | |
|---|---|---|
| Radiation | In the zenith | Near the horizon |
| UV-B | 1·54 | 0·004 |
| UV-A | 47 | 2·1 |
| | (h = 90°) | (h = 5°) |

to specify the radiation incident on the frontal or dorsal aspect of the human body (total surface 2 $m^2$).

The sun rises to its zenith on the spring equinox when it stands 40° above the horizon; summer solstice when it is 63·5° above the horizon; autumn equinox at 40° above the horizon; and winter solstice at 16·5°. These values apply generally to Central Europe. Solar intensities are smallest in winter because the aerial path of the solar rays is longest. Hence, the danger of causing a solar carcinoma is least during the winter months.

Aerial pollution is another contributory factor to shifting the shortwave limit of atmospheric transparency towards longer waves. Pressure gases—mostly dichlorofluoroethane—as used in spray canisters whether for hair fixers or paint rise not much above 2000 m into the air adding to the formation of mist and cloud (Summer, 1971a). The exhaust gases of modern supersonic aircraft flying at 20,000 m altitude and higher are causing the reverse effect. The exhaust gases, released close by, even into the ozone layer, are reacting chemically with the ozone, thus reducing its protective potential and thereby enabling the short biocidal solar rays to reach the surface of the earth.

The value 292·5 nm quoted as the lower limit of atmospheric transparency is found today only in places far removed from human occupation (air pollution) and at high altitudes. The latter condition is governed by the fact that the air mass (m) on top of a high mountain peak is less than over a low lying plain. With the sun in its zenith the increase in solar intensity with increasing altitude is shown in Table 5. The lesser increase in solar UV-A intensity is in consequence of the low-loss transmission through the atmosphere. The values in Table 5 are of importance to heliotherapy.

The 'optical window' in the earth's atmosphere allows the incidence of ultraviolet and light rays at an intensity of 1000 W $m^{-2}$. Cosmic rays and X-rays of cosmic origin penetrate our atmosphere practically without any loss, but at very low intensities, $10^{-13}$ W $m^{-2}$ as an average, and infrared rays produce 10 million times more intense effects but still achieve an average value of no more than $10^{-6}$ W $m^{-2}$.

The carcinogenic spectrum of solar rays reaching the surface of the earth comprises the range 292·5–320 nm. The lower limit applies to the absence of atmospheric pollution and high altitudes. Air pollution is not always due to human occupation. Conifers give off 1000 million tons of terpenes

TABLE 4. Values of the solar constant

| | | |
|---|---|---|
| Extraterrestrial | 2 gcal $cm^{-2}min^{-1}$ | = 1360 W $m^{-2}$ |
| At 3500 m altitude | 1·6 | = 1088 |
| At sea-level | 1·4 | = 952 |

1

TABLE 5. Differential in solar intensity with altitude

| Altitude (m) | Differential in intensity (%) | |
|---|---|---|
| | Solar UV-B | Solar UV-A |
| 1000 | 20 | 17 |
| 2000 | 35 | 27 |
| 3000 | 50 | 34 |
| 5000 | 70 | 44 |

$C_{10}H_{16}$ globally per annum. These aromatic hydrocarbons of vegetable origin concentrate, logically. over the continental earth masses while the air over the oceans is practically free from terpenes, Although the average concentration is only $10^{-9}$ the molecules of terpenes scatter the short (blue) rays of sunshine thus appearing as a bluish haze over high mountains, especially over the range known for that reason as the Blue Mountains (Summer, 1971b).

In urbanized districts populated by large numbers the atmospheric pollution due to solid particles (smoke, dust) and aerosols (fog, mist, steam) may easily raise the lower limit of atmospheric transparency (292·5 nm) beyond the upper limit of carcinogenic radiation (320 nm). The reason why 320 nm is considered the upper limit of carcinogenic radiation is that the quantum energy of this radiation is $6{\cdot}2\times10^{-19}$ J and that energies below $6\times10^{-19}$ J cannot induce biological changes. This is certainly the reason, too, why the upper limit of erythemogenic radiation occurs at the same wavelength. The upper layers of the epidermis absorb 87% at 292·5 nm, and 76% at 320 nm, transmitting 13% and 24% respectively to the deeper layers right down to the stratum basale: 13% of $6{\cdot}8\times10^{-19}$ J $= 0{\cdot}88\times10^{-19}$ J; 24% of $6{\cdot}2\times10^{-19}$ J $= 1{\cdot}49\times10^{-19}$ J may develop cancerogenic activities in the stratum basale.

The cancerogenic spectrum is quoted by Magnus (1972) as being located between 240 and 320 nm while Forbes (1972) considers the upper limit to be reached at 315 nm which provides a slightly more energetic radiation, namely $6{\cdot}3\times10^{-19}$ J. The portion of sunshine capable of inducing cancer of the skin is about 1 mJ $s^{-1}$ $cm^{-2}$ inclusive of all losses by scattering and reflexion. Related to one-half the body surface, this value is 10 J $s^{-1}m^{-2}$ = 10 W $m^{-2}$.

Viewing it from the physical point of view, the cause of a photocarcinoma is not only the quantity of absorbed energy (Grotthus-Draper law) but also its quality, i.e. the wavelength at which the energy radiates. Epstein (1972) reports that 0·449 J $cm^{-2}$ = 4490 J $m^{-2}$ at wavelengths below 320 nm effectively suppresses mitosis and causes 'interruption of the cell cycle, and inhibition of DNA, RNA and protein synthesis in the mammalian epidermis *in vivo*'.

The biological processes occur thus: 72 h after the end of irradiation, the rate of mitosis as well as that of basal cell proliferation increases beyond its standard values; connected with this is an acanthosis of the epidermis. Together with the appearance of carcinogens in the irradiated skin, the above outlined processes are the cause of a solar carcinoma. The formation of cancerogens in the skin after ultraviolet irradiation has been assumed many times and sometimes even accepted, but it has now been proven that they are certain steroid-hyperperoxides (Tronnier, 1977).

An important contributory factor to a solar carcinoma is the total time the person has been exposed to the sun. In the case of occupational necessity the period of exposure may be of many years duration. This is so in highland farmers, sailors and other people with similar occupations. The irradiation dose is calculated after the general formula: intensity of the radiation × exposed skin area × the

TABLE 6. Relative photosensibility of white man (Ellinger, 1941)

| | | | | | |
|---|---|---|---|---|---|
| Falling | April | 100 | October | 85 | Rising |
| | May | 83 | November | 84 | |
| | June | 23 | December | 81 | |
| | July | 41 | January | 87 | |
| | August | 36 | February | 57 | |
| | September | 32 | March | 92 | |

duration of exposure. Assuming the skin areas exposed to the sun being the nape of the neck: 100 $cm^2$; face: 300 $cm^2$; hands: 300 $cm^2$; and the duration of exposure 8 h/day for 15 years, the carcinogenic dose is:

$$\underset{\mathrm{mJ\,s^{-1}cm^{-2}}}{1} \times \underset{\mathrm{cm^2}}{100} \times \underset{\mathrm{a}}{15} \times \underset{\mathrm{d}}{360} \times \underset{\mathrm{h}}{8} \times \underset{\mathrm{s}}{3600} = 15600000\ \mathrm{J} = 15{\cdot}6\ \mathrm{MJ}$$

for the neck, and three times this amount for the face and hands. Biological processes are at first an inflammation of the exposed skin area, followed by carcinomata, telangiectases and senile elastosis (keratosis senile). Basal cell carcinomas, too, may appear. As concerns data resulting from animal experiments, the warning should be heeded that 'to reach the doses labelled cancerogenic in animal experiments man would have to be exposed to massive doses of radiation daily for 15–30 years (Tronnier, 1977).

In addition to the fixed data of cosmic—to be more precise, of solar—conditions concerning the intensity and spectral range of the sun's radiations, there are the variations in human properties causing differences in reactions to incident solar energy. Man's physiological reactions are affected by external (environmental) as well as by internal conditions which in themselves are variable. Environmental conditions include the seasonal changes of solar incidence causing changes in man's photosensibility (Table 6) and endogenously conditioned variations in photosensibility that occur in pregnancy (Table 7).

Dark skinned people are well protected against solar carcinogenic radiation. It is not only that the pigmented skin affords an efficient defence against the intense solar rays, but also that the people

TABLE 7. Relative photosensibility during pregnancy of white woman (Dietrich, 1928)

| | | |
|---|---|---|
| | Month 1 | 100 |
| | Month 2 | 100 |
| Rising | Month 3 | 140 |
| | Month 4 | 250 |
| | Month 5 | 270 |
| | Month 6 | 280 |
| | Month 7 | 300 |
| | Month 8 | 160 |
| | Month 9 | 200 |
| | Month 10 | 150 |
| | Post partum | 100 |

are spared the physiological shock of suddenly being exposed to a degree of solar intensity to which they are neither accustomed nor into which they were born. White man suffers severely from that shock against which he cannot muster sufficient natural protection. The absence of this physiological shock explains that among the Hunza, a dark skinned people living on the high plateau of the Himalayan mountains (5000 m above sea-level) north of Kashmir, cancer is not known at all (Peeters & Verhasselt, 1977). This is the more important since the coincidence of great altitude and absolute purity of air cause a 70% increase (cf. Table 4) in intensity of carcinogenic radiation. The Hunza are, thus, exposed to $17\ \mathrm{J\,s^{-1}m^{-2}}$ which makes the exposure to cancerogenic radiation during a lifetime of, say, 50 years:

$$\underset{\mathrm{J\,s^{-1}m^{-2}}}{17} \times \underset{\mathrm{m^2}}{1} \times \underset{\mathrm{a}}{50} \times \underset{\mathrm{d}}{360} \times \underset{\mathrm{h}}{8} \times \underset{\mathrm{s}}{3600} = 8800\ \mathrm{MJ}$$

## ARTIFICIAL RADIATION

The therapeutic application of artificially generated ultraviolet radiation has brought the question of photocarcinogenesis into the limelight as there seems to be a risk to both patient and personnel. It has been the custom to discuss both risks under the same heading, but this leads to an erroneous result. Consider the physical concept of sources of ultraviolet radiation. Their cancerogenic output depends both qualitatively and quantitatively on basic design and may have varying values within a very wide range. Modern equipment emits continuous radiation in various parts of the ultraviolet spectrum ranging from 160 nm to 380 nm. The gamut of the emission spectrum depends on both the material of the envelope and the complexity of the discharge arc. A representative UV generator of the polykymatic type produces an intensity of $3{\cdot}66\ \mathrm{mJ\,s^{-1}cm^{-2}} = 36{\cdot}6\ \mathrm{J\,s^{-1}m^{-2}}$ at standard distance of 1 m*. Total exposure varies from 160–200 min depending on type and severity of the skin disorder. In the calculation below an irradiation time of 180 min is used as an average figure. Of the $36{\cdot}6\ \mathrm{J\,s^{-1}m^{-2}}$ emitted within the continuous spectrum (effective from 295 to 335 nm) $27{\cdot}3\ \mathrm{J\,s^{-1}m^{-2}}$ are within the cancerogenic section 295–320 nm. This yields a photocancerogenic dose of

$$\underset{\mathrm{J\,s^{-1}m^{-2}}}{27{\cdot}3} \times \underset{\mathrm{m^2}}{1} \times \underset{\mathrm{min}}{180} \times \underset{\mathrm{s}}{60} = 294848\ \mathrm{J} = 0{\cdot}295\ \mathrm{MJ}$$

It is the carcinogenic dose for the frontal or dorsal aspect of the whole body.

## COSMETIC RADIATION

Exposure to ultraviolet radiation without specific direction by a dermatologist should be given more consideration than to the inquiry into possible cancerogenic risks of properly administered phototherapy. The mainly cosmetic effect of irradiation by a solarium may constitute such a risk if the sources of ultraviolet energy are not properly selected for the purpose.

In many cases the sources of UV rays incorporated in a solarium emit a spectrum (line spectrum) from 250 nm upwards well into the infra-red. This includes nearly all cancerogenic rays which are well transmitted through the glass envelope of UV generators. Hard glasses of the vycor and similar types transmit 80% of radiation at 250 nm and 90% throughout the UV-B and UV-A range. Solaria contain up to eight ultraviolet lamps at 250 W each, emitting $8 \times 140\ \mu\mathrm{J\,s^{-1}cm^{-2}} = 11{\cdot}2\ \mathrm{J\,s^{-1}m^{-2}}$ cancerogenic energy at 1 m standard distance. People are enjoying 'sunshine' for about 1 h/day, but

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* Data published by courtesy of the manufacturers G. Saalmann, D-49 Herford.

TABLE 8. Comparison of cancerogenic radiation from various sources

| Source | Absolute (MJ) | Relative |
|---|---|---|
| Solarium | 29 | 98 |
| Sun | 15·6 | 53 |
| Phototherapeutic lamp | 0·295 | 1 |

often more, continuing the habit for perhaps 2 to 3 years until the novelty has worn thin, and fashion has found a new fad. The total cancerogenic dose received during this period is:

$$\underset{\mathrm{J\,s^{-1}m^{-2}}}{11{\cdot}2} \times \underset{\mathrm{a}}{2} \times \underset{\mathrm{d}}{360} \times \underset{\mathrm{h}}{1} \times \underset{\mathrm{s}}{3600} \times \underset{\mathrm{m^2}}{1} = 29030400\ \mathrm{J} = 29\ \mathrm{MJ}$$

Comparing the amounts of cancerogenic artificial radiation (phototherapeutic UV generators and solarium) incident upon either the frontal or dorsal aspect of the body with the solar rays striking but the neck or face of a person exposed to it, the data in Table 8 are obtained.

It should be borne in mind that the irradiated body surface in the case of both solarium and lamp is 1 $\mathrm{m^2}$, but in the case of the sun is 0·01 (0·03) $\mathrm{m^2}$.

The danger of 'burning' a person by excessive ultraviolet irradiation is very real, though the degree of 'burning' is controlled by several individual factors which vary from person to person and with the bodily condition at the time of being irradiated. A natural protective factor is pigmentation which prevents the offending ultraviolet rays from entering the skin.

Personnel in clinics and consulting rooms of dermatologists are exposed to ultraviolet radiation only for 1–2 min per patient—unless they are careless or if the positioning of patient and ultraviolet generator requires the presence of the assistant in the irradiated area for a few moments. Only hands and neck of the assistant are exposed to radiation if neither is hidden under protective clothing. Assuming six patients per hour in an 8-h stretch of duty makes for a total exposure of $6 \times 8 \times 2 = 96$ min/day. The exposed surface of the unprotected skin will in no case exceed 500 $\mathrm{cm^2}$ making the dose received by the assistant:

$$\underset{\mathrm{mJ\,s^{-1}cm^{-2}}}{2{\cdot}73} \times \underset{\mathrm{min}}{96} \times \underset{\mathrm{s}}{60} \times \underset{\mathrm{cm^2}}{500} = 7862400\ \mathrm{mJ} = 7862\ \mathrm{J}$$

This is roughly 1/40 of the patient dose. If the assistant goes sunbathing and lies in the sun for 3 h—by no means an excessive exposure—the solar cancerogenic dose received is:

$$\underset{\mathrm{mJ\,s^{-1}cm^{-2}}}{1} \times \underset{\mathrm{h}}{3} \times \underset{\mathrm{s}}{3600} \times \underset{\mathrm{cm^2}}{10000} = 108000000\ \mathrm{mJ} = 108000\ \mathrm{J} = 0{\cdot}108\ \mathrm{MJ}$$

which is fourteen times the dose received daily as an assistant at the skin clinic.

Clothing offers protection from the incidence of UV rays only to a limited degree (Table 9). Material and weave affect the restrictive power of the fabric.

The risk of photocarcinogenicity as a late after-effect of phototherapy, not photochemotherapy, is negligible if the treatment is directed by a dermatologist (Tronnier, 1977). The relative safety from carcinogenicity is based on the moderate number of sessions, the short duration of each exposure, the exact dosage, and the absence of concomitant medication (sensitization).

TABLE 9. Ultraviolet transmissivity of fabrics (Summer, 1962)

| Material | Transmissivity (%) |
|---|---|
| Muslin (white) | 50 |
| Cotton voile | 40 |
| Silk stockings | 25 |
| Cotton stockings | 18 |
| Rayon stockings | 10·5 |
| Satin (beige) | 10 |
| Wool stockinet | 2 |
| Flanelette | 0·3 |
| Poplin | 0 |

'Safe doses' may be regarded by some as a utopian formulation. The American Medical Association (AMA) compiled a list of Maximum Safe Exposures some 25 years ago based on the 'safe dose' of 216 $\mu$W min$^{-1}$ cm$^{-2}$ (130 Jm$^{-2}$) at wavelength 253·7 nm (Summer, 1962). Safe limits have been re-evaluated. The American Conference of Government Industrial Hygienists has published safe limit doses for occupational exposure. The data have been adopted by United Kingdom experts, i.e. by the National Radiological Protection Board (NRPB), and by the International Radiation Protection Association (IRPA) (1977).

The similarity of psoriatic lesions and photocarcinomata—in either case greatly increased mitosis and cell proliferation—has often been the cause of anxious thoughts that psoriasis might be followed by skin cancer. Fortunately, no such risk has been observed.

The absence of skin carcinomata in a psoriatic person has caused Professor Sam Shuster to propose his hypothesis that psoriatic skin is almost incapable of metabolizing precancerogenic substances. This, Shuster thinks, is a consequence of the greatly reduced activity of arylhydrocarbon hydroxylase (AHH). He says: 'We must therefore consider the possibility that psoriasis has persisted because it offers a genetic advantage—namely, the decreased activity of psoriatic skin—which reduces the incidence of cancer of the skin and perhaps of other organs (Shuster, Chapman & Rawlins, 1979).

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*British Journal of Dermatology* (1980) **102,** 621.

# *Correspondence*

## Reticulate urticarial erythema in hereditary angio-oedema

SIR, In his recent article on reticulate erythema as a prodrome in hereditary angio-oedema (HAE), Williamson (1979) describes a peculiar skin eruption occurring in several members of a large family affected with HAE.

We would like to relate our own observation of a similar case. Full details of the diagnosis, the clinical course and the treatment of this patient have been published (Hintner *et al.*, 1979). In our patient there were attacks of a peculiar reticulate urticarial erythema that had been recurring at 3-week intervals almost since birth; after 1–3 days the skin eruption was followed by abdominal pain and vomiting. The erythema always subsided within 1–2 days together with the gastrointestinal symptoms. Attacks of typical angio-oedema occurred independently. The skin eruption did not appear in his mother who has HAE, nor is it reported to have been present in his sister, who had died from laryngeal oedema some years previously.

Clinically, the skin eruption of our patient is characterized by a bizarre, polycyclic configuration involving most of the skin surface. It looks like regressing urticaria; its configuration changes within fractions of an hour and the skin between the linear weal-like lesions appears clinically normal (Fig. 1). Upon treatment with Danazol, as detailed (Hintner *et al.*, 1979), the attacks of angio-oedema, the skin lesions and the gastrointestinal attacks subside.

The features of our patient's skin eruption are reminiscent of the reticulate erythema described by Williamson (1979), which, even though occurring independently from angio-oedema of the skin, was also a prodrome to abdominal symptoms. However, there are also differences: although the family is small (only three members are known to have or have had HAE) and thus not very informative, there is no evidence for a familial occurrence of this peculiar eruption and perhaps more importantly, Williamson describes the lesions as annular and reticulate erythema, whereas in our patient they were urticarial in character.

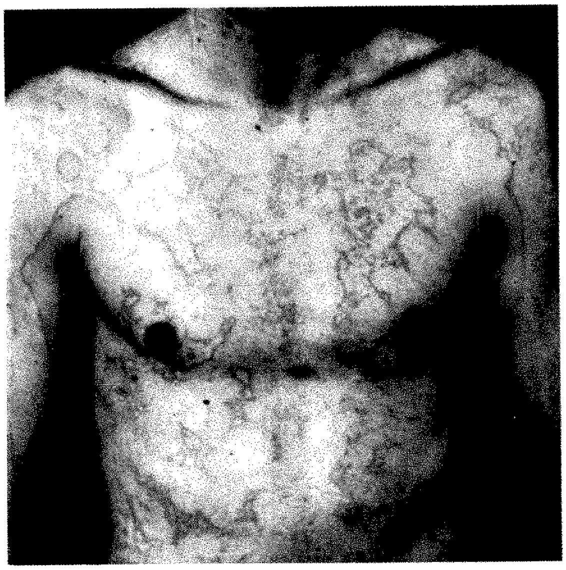

FIGURE 1. Reticulate, urticarial erythema in a patient with HAE 24 h before onset of colicky abdominal pain.

In their series of patients with HAE, Gelfand *et al.* (1976) found asymptomatic erythematous eruptions in 26% of the cases. They did not describe the erythemas in more detail, but emphasized the lack of a weal-like quality which separates these rashes from the eruption of our patient.

It seems to us that the most logical conclusion to be drawn from these different observations is that the process giving rise to angio-oedema can also affect superficial vessels of the skin and then produces erythemas or weals. What additional genetic, immunological or individual factors play a role in the appearance of reticulate erythemas or weals in HAE patients, however, remains a matter for speculation.

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## 'Methodology' versus 'method'

SIR, Contributors to the Journal are increasingly, and wrongly, using 'methodology' (the science of method) when they mean 'method', (the procedure for attaining an object). If we must use neologisms we should at least know their exact meanings. Wittgenstein would not have approved!

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BENJAMIN PORTNOY

*British Journal of Dermatology* (1980) **102,** 623.

# Book Reviews

**Histologic Diagnosis of Inflammatory Skin Diseases.** A. BERNARD ACKERMAN (1978) Philadelphia: Lea and Febiger. Pp.863.

This book is concerned with the histological diagnosis of inflammatory skin diseases and their recognition by pattern rather than by presumption of a cause or mechanism. Since the aetiology and pathogenesis of most skin disorders are largely unknown and as at present there is no orderly approach to the histological diagnosis of inflammatory skin diseases, the author is justified in putting his method of pattern analysis and producing this unusual book.

The first four chapters are concerned with the structure and function of the skin and of the inflammatory cells, definition of terms and with biopsy techniques. These can be found in other text-books of dermatopathology, but they are well written, accurate and contain many excellent illustrations. However, for me, the book really starts at chapter five in which the author delineates nine major patterns of histological expression of inflammatory skin diseases. These can be recognized with the scanning power of the microscope. As one reads this and the subsequent chapters one suspects that most dermatopathologists use this 'pattern method' whether they realize it or not. Like Bernard Ackerman I too was greatly influenced by Wallace Clark and can recognize much of his inspired teaching in this book. Inflammatory skin diseases can certainly be classified on the basis of distinctive histological patterns.

The book is lavishly produced and is written in a clear but somewhat didactic style. At the beginning of each section the main histological features of the condition described are separately summarized and this is of considerable use to the less experienced dermatopathologist. The enormous experience and knowledge of the author are evident throughout the book and there are very few errors to be found. Most of the black and white photomicrographs are of good quality though some of the lower power ones are somewhat indifferent and are rather fuzzy. In addition there are several good colour plates. There are quite a number of schematic drawings some of which do not contribute very much to the further understanding of the disease that they are meant to illustrate. Another unusual feature is that there are no references to the literature in the text and as a consequence the book is even more didactic and very much a personal account. The final chapter has a very useful check list that would certainly help the beginner in dermatopathology in interpreting histological sections of inflammatory diseases of the skin. Finally there is a very good index which is most necessary for finding one's way round this book.

It is a rather expensive tome and has the form and dimensions of an art book that would certainly grace a coffee table in the home. However, I think that this is a book that should be purchased for both hospital and departmental libraries and one that should be available for the histopathologist at work in the reporting room.

S.S.BLEEHEN

**Immunological Aspects of Contact Sensitivity. Monographs in Allergy, 15.** L. POLAK (1980) Basel: S.Karger. Pp.170, 47 figures, 24 tables.

It would be a great pity if, in the discipline of dermatology where allergic contact dermatitis is part of our 'bread and butter', this excellent treatise is not read as widely as it should be. The monograph is a well written, comprehensive review, containing over 800 references, of current understanding of the immunology of contact allergy. The author, who himself has contributed much to the subject, provides a detailed yet lucid account of the basic pathogenetic mechanisms operating in contact sensitivity.

The text is broadly divided into two halves. The first half, after a brief introduction, follows the familiar primary response with the afferent limb, lymph node and efferent limb phases, and the secondary (challenging) response. There are brief discussions on the still undefined role of the basophil in cutaneous hypersensitivity and the differences that exist between tuberculin-type and contact allergy. The latter half of the book concentrates on the more difficult and elusive problems of control mechanisms. Particular reference is paid to immunological tolerance, suppressor cells and desensitization.

Most of the work in this field has been, of necessity, based on animal (particularly guinea-pig) studies. However, despite this and the accompanying caveat with regard to extrapolation to man, this summary is valuable and thought provoking. I am sure that all dermatologists should read it.

R.J.G.Rycroft

**A Colour Atlas of Pathogenic Fungi.** D. Frey, R.J.Oldfield and R.C. Bridger (1979) Wolfe Medical Publications: London. Pp. 168, approx. 400 colour plates. Price £16.

This is an extremely attractively produced colour atlas. With a page size of 19 × 26 cm it is larger than the earlier Wolfe Colour Atlases and this allows the numerous colour plates to be arranged sensibly with annotations adjacent to them. A wide range of fungal pathogens is included with photographs of colonial appearances suitably labelled as to age and media along with photomicrographs to illustrate microscopic features required for identification. Direct microscopy of skin, hair and nails is generally excluded and the histopathology of fungal disease is recorded only insofar as it relates to fungal morphology within the tissues. The quality of these colour reproductions is, in general, excellent but a curious omission is the absence of any views of the reverse side of fungal colonies to show pigmentation, although the text does, of course, mention this.

The choice of fungi selected is somewhat arbitrary, for example, *Microsporum boullardii* is included with the comment, 'Single isolate from the African Republic of Guinea, geophyllic, pathogenicity not reported', but *Scopulariopsis brevicaulis* receives no mention and among the Aspergilli, only *Aspergillus fumigatus* and *Aspergillus flavus* are included. The omission of *Trichophyton mentagrophytes var. nodulare* is also annoying. This reviewer would willingly have sacrificed a few of the rarities for more examples of the variations seen among common species.

The text is very brief and, although supplemented by several tables in an appendix at the rear, this book must not be thought of as a manual for identifying fungi. It is, as its title states, a picture book and as such, it will make a useful addition to the laboratory book shelf. I think it is reasonably priced at £16.

S.O.B.Roberts

**The Mast Cell: Its Role in Health and Disease.** Edited by J.Pepys and A.M.Edwards (1979) Tunbridge Wells: Pitman. Pp. 873, illustrated. Price £16.50.

The mast cell usually appears to the clinician as a trouble-maker, a keg of high explosive waiting to go off. One can but think that it ought to have evolved to have a bigger and better role than to be part of the effector system, together with IgE, for getting rid of intestinal parasites. In this volume Padawer suggests that the main function of mast cell granules is carried out intracellularly—without degranulation, an event which nature may never have intended to be very useful.

This 873 page soft back volume represents the proceedings of an excellent symposium held in Davos in April 1979, sponsored by Fisons. The aim was to bring together people from very diverse disciplines, but who share an interest in the mast cell and its role in diseases, or in the therapeutic efficacy disodium cromoglycate.

There are 170 pages on the basic anatomy, physiology, biochemistry and pathology of the mast cell; then a section on the still rather obscure mode of action of cromoglycate; then follow sections on respiratory disease, food allergy, eye diseases, skin diseases, inflammatory bowel disease and ENT diseases. There is, of course, a considerable emphasis on cromoglycate, a drug which remains quite remarkable for its efficacy and safety. However, the majority of the papers deal with the fundamental pathology of the mast cell or of such diseases as atopic dermatitis. Although we are told that sixty-five companies have taken out some form of patent in this area, disodium cromoglycate still remains supreme and virtually unchallenged in regular clinical practice.

For skin diseases there is encouragement for using oral cromoglycate to control the symptoms of systemic mastocytosis (there may be a delay of up to 3 weeks before benefit is apparent), to control food allergies including urticaria and cases of idiopathic urticaria with positive challenge tests to food additives, and to treat atopic dermatitis. There is some conflict about the efficacy of topical DSCG in atopic dermatitis.

Altogether there are 129 papers from 344 authors.

The editors clearly decided to have this book as a faithful recording of a meeting rather than a textbook on the mast cell consecutively covering all aspects. The original commissioning of the papers made sure of a comprehensive cover but there is inevitably some overlap, conflict, uneveness. To have got this nicely produced book out within six months of the meeting is indeed worthy of congratulation to both editors and publishers. There is much in it to be gleaned by dermatologists whether their interests be academic or clinical.

R.H.CHAMPION

**International Congress Series 451. Proceedings of the XVth International Congress of Dermatology, Mexico, 16–21 October 1977.** Edited by ANTONIO GONZALEZ-OCHOA, LUCIANO DOMINGUEZ-SOTO AND YOLANDA ORTIZ (1979) Amsterdam: Excerpta Medica. Pp. 860, B/W illustrated. Price U.S. $131.75, Dfl. 270.

The writer's heart sank when he was asked to review this volume since the published proceedings of previous international congresses of dermatology have generally been large, unattractively presented and deadly dull. In fact, his pessimism was unjustified and the publishers, Excerpta Medica and the editors of this record of the highly successful and well organized XVth Congress in Mexico City in October 1977 are to be congratulated on their readable, visually attractive and manageable single volume record of this meeting.

The great majority of special lectures and invited contributions to symposia are recorded together with many free communications and summaries of workshops. Most contributors have included references, and comprehensive subject and author indices help to make this volume a useful aide memoire for those who were at the Congress and a worthwhile reference source for other readers.

An analysis of the countries of origin of the contributors shows how difficult it is to make such meetings truly international, rather than regional. The vast majority of published contributions are from Europe and the Americas and Japan, with very little from Africa, the rest of Asia, or the Soviet bloc of countries. Perhaps the decision to publish only in English contributed to this uneven international effort.

The appearance of this volume justifies the last minute decision of the International Committee just before the XVth Congress to publish these proceedings, reversing their decision not to do so taken after the 1972 Venice-Padua Congress. It would have had even more impact had it been possible to shorten the 2 year delay between the Congress and publication of this book.

HARVEY BAKER

*British Journal of Dermatology* (1980) **102,** 626.

# News and Notices

### Aspen Summer Skin Seminar

This 5-day Course will be held at The Given Institute of Pathobiology, Aspen, Colorado, 16–20 July 1980.

It will cover a broad range of topics related to the clinical practice of dermatology, including acne, sunlight, pruritus, oral ulcers, paediatric skin diseases, systemic diseases, immunology and fungal, bacterial, and viral infections of the skin.

Further information may be obtained from: The Office of Postgraduate Medical Education, The University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, CO 80262, U.S.A.

### Molecular Basis of Dermatologic Disorders

An advanced study program (workshops and seminars) on the 'Molecular basis of dermatologic disorders' under the sponsorship of the North Atlantic Treaty Organization (NATO) Advanced Study Institutes (ASI) Program will be held 15–24 September 1980, at Corfu, Greece.

For participation and further information write to: Dr M.A.Pathak, Director NATO ASI Program, Mass. General Hospital, Dept. of Dermatology, Boston, MA 02114, U.S.A.

### Medicine and Mycology

A course on common and important fungal infections will be held at Fitzwilliam College, Cambridge, 24–26 September 1980.

It should prove valuable for clinicians of various disciplines but particularly dermatologists. The course is not primarily concerned with the technical aspects of medical mycology or of the identification of fungi. Medical microbiologists will find that the course emphasizes the definition of the clinical problem and the role of the laboratory in diagnosis and management.

Further details and an application form may be obtained from: The Conference Secretary (Medicine and Mycology), Postgraduate Medical Centre, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

## APPOINTMENTS

### Barnet Area Health Authority

Dr Helena M.Barnes has been appointed to the post of Consultant Dermatologist at Edgware General Hospital, London and Barnet General Hospital.

She graduated MBBS from the Royal Free Hospital School of Medicine in 1968 and obtained her MRCP in 1971. Following general medical posts at the Central Middlesex Hospital, London and in Welwyn Garden City, she was appointed Registrar in Medicine at the Hammersmith Hospital. In 1972 she became Dermatology Registrar and then Senior Registrar at the Royal Free Hospital, London. Lately this post has been part-time.

Her publications have been mainly of a clinical nature and she has also published several papers on archaeological remains.

## St John's Hospital for Diseases of the Skin

Dr W.A.D.Griffiths has been appointed Consultant Dermatologist to St John's Hospital for Diseases of the Skin, London. Dr Griffiths studied medicine at Emmanuel College, Cambridge and St Thomas's Hospital, London, qualifying in 1965. He was Senior Registrar at St John's Hospital and spent a year at Pahlevi University, Shiraz, Iran. In 1975 he was appointed Consultant Dermatologist to the Liverpool Area Health Authority (Teaching) and the Mersey Region.

His publications reflect his interest in clinical dermatology. He obtained his M.D. for his study of 'Pityriasis rubra pilaris'.

## Errata

In the paper entitled 'Effects of corticosteroids on the proliferation of normal and abnormal human connective tissue cells' by G.C.Priestley and J.C.Brown (*British Journal of Dermatology*, **102**, 35) the symbols at the bottom of Figure 1b should be solid circles (and not open circles as shown).

In the paper 'The Ehlers-Danlos syndrome: an analysis of the structure of the collagen fibres of the skin' by C.M.Black *et al.* (*British Journal of Dermatology*, **102**, 85) the labelling on Figure 7 should read $\gamma$III opposite the third dark band in Track 1 and $\alpha$III should be ignored.

## NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

**Manuscripts** should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$ g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$ g) | $\mu$g | cubic millimetre(s) | mm$^3$ | micrometre(s) | $\mu$m |
| nanogram(s) ($10^{-9}$ g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$ g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hour(s) | h | milliequivalent | mmol | | |

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

**Illustrations** should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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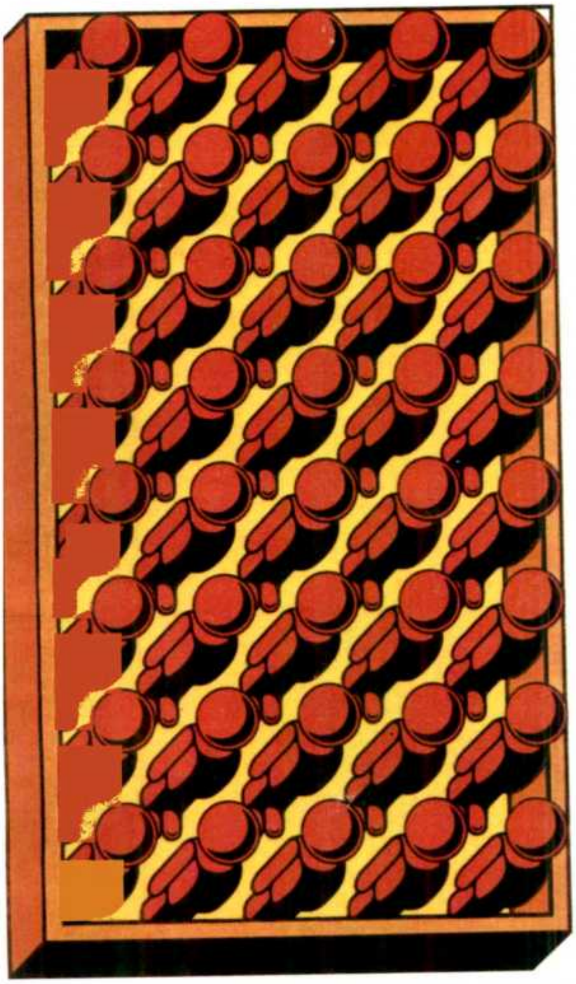

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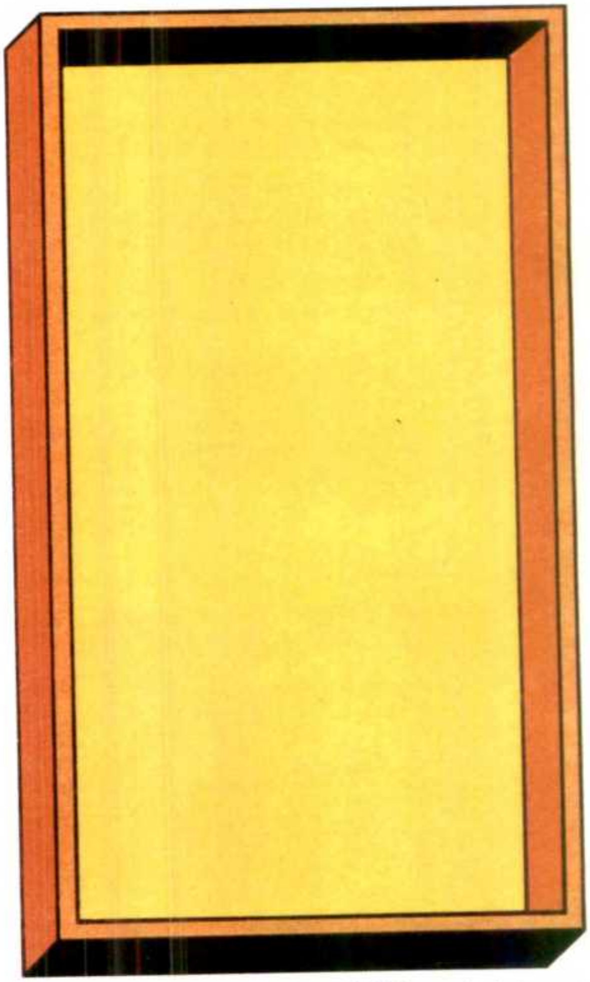

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**References:**
1. *British Journal of Dermatology*, 1979, **100**, 475

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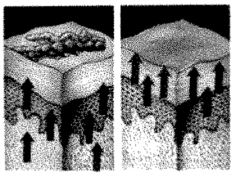

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### Prescribing information

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Doses should be taken after meals for maximum absorption

*Adults*. normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily

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**References:**
**(1) Grupper, Ch. and Avram, A. Proceedings of VIth ISHAM Congress. Tokyo. June 1975. p. 162**
**(2) Schwartz, K. J. et al Deutsch. med. Wochenschr. 1975.** *100*, **1497.**
**(3) Gisslen, H. et al Curr. ther. Res., 1977,** *21*, **681**
**(4) Dorn, M. et al Munch. med. Wschr. 1975** *117*, **687**

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*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol. 33 No. 4, 106.

**Presentation** A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, u.s.p. 0.5% in a greaseless vanishing-cream base. The cream is smooth[redacted]steroid anti-inflammatory therapy for psoriasis. **Dosage and Administration** Apply two to four times daily massaging vigorously into the affected areas. **Contra-indications and Warnings, etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion.

of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in lar[redacted] £2.29 for 45gm. **Product Licence No.** 0036/0026

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re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
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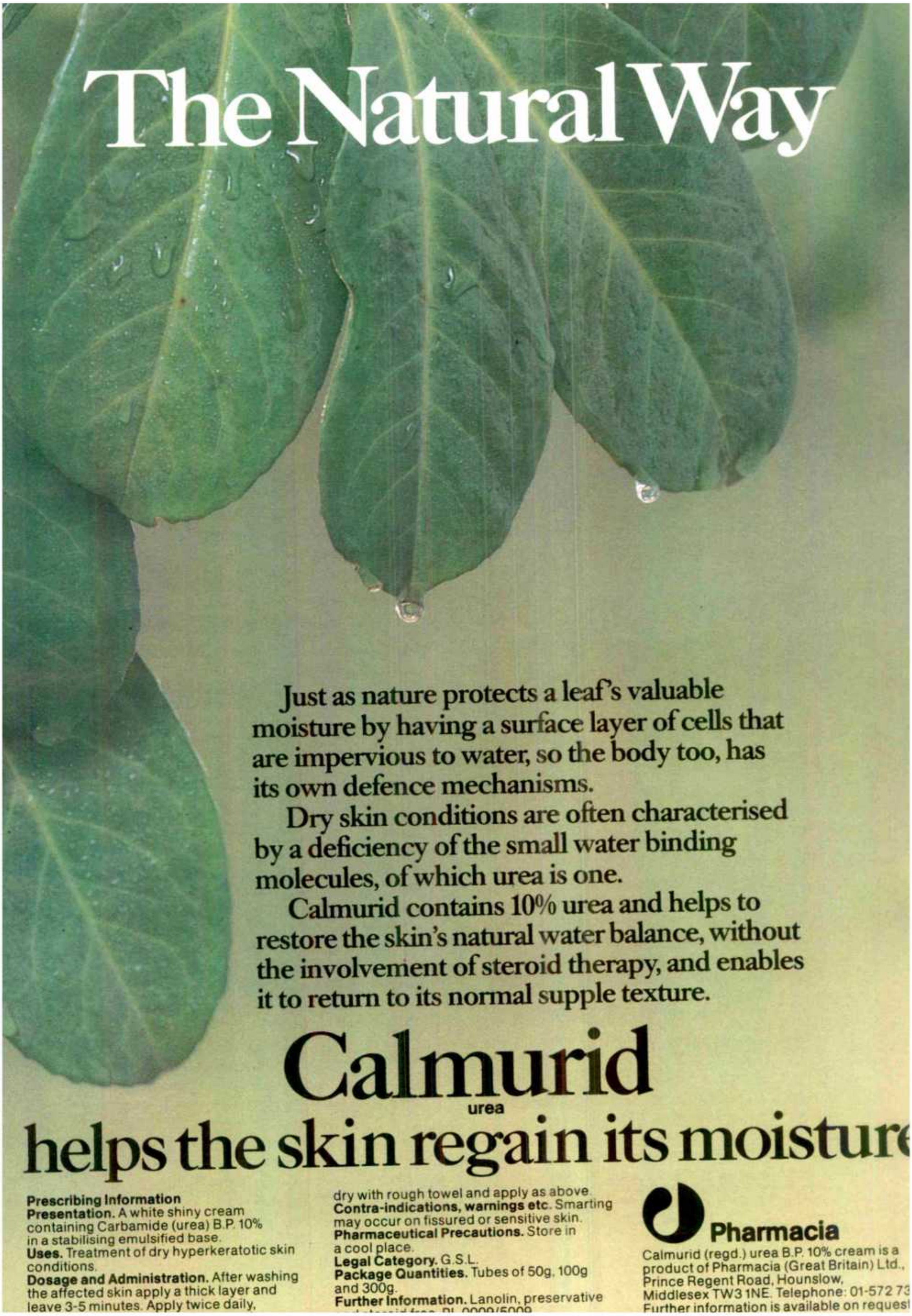

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urea
**helps the skin regain its moistur**

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**Contra-indications, warnings etc.** Smarting may occur on fissured or sensitive skin.
**Pharmaceutical Precautions.** Store in a cool place.
**Legal Category.** G.S.L.
**Package Quantities.** Tubes of 50g, 100g and 300g.
**Further Information.** Lanolin, preservative

**Pharmacia**

Calmurid (regd.) urea B.P. 10% cream is a product of Pharmacia (Great Britain) Ltd., Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 73
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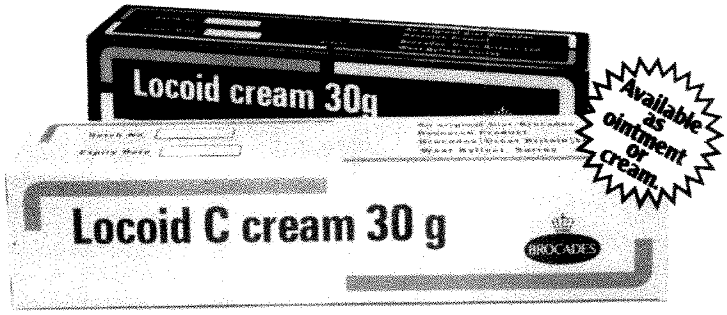

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**References:**

(1) Amer. Pract. & Digest of Treat. (1955) 6, 8
(2) Transact. New York Acad of Sci. (1955) 10, 161
(3) Arch Derm (1972) 106, 335
(4) Rocky Mtn. Med. J. (1955) 52, 6

**Prescribing Notes**

**Dosage & Use.** Apply to the affected part two to four times a day until the infection is resolved. If the underlying condition persists treatment may be continued for up to 14 days and no longer. No more than 60g may be applied during this period. Do not use under occlusion in the presence of infection.
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**Cost** Basic NHS price £2.39 for 30g
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**Presentation** Sterile, straw-coloured spherical beads of dextranomer of 0.1-0.3 mm diameter, packed in plastic castors or single-use sachets.
**Uses** A dressing for moist, small area burns, wounds and indolent ulcers, whether clean or infected.
**Dosage and administration** A 3 mm layer of Debrisan should be sprinkled on to the wound and kept in place by a pad of lint or a perforated plastic sheet. Debrisan is hydrophilic and the tissue exudate is drawn up into the layer. The Debrisan should be renewed before saturation occurs. Depending on the rate of exudation this may be necessary from one to five times a day. Twice daily is usually adequate. When Debrisan is changed the old material is readily rinsed off with water or saline and new material may then be sprinkled on to the wound.
Full instructions for use are enclosed with each pack. Treated in this manner the wound will remain soft and pliable during healing.
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1. Do not leave Debrisan for more than 24 hours on wounds with a very low exudation rate as it may dry and form a crust which may be difficult to wash off.
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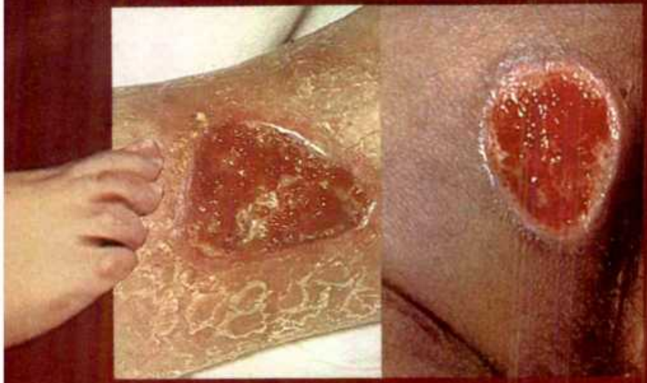
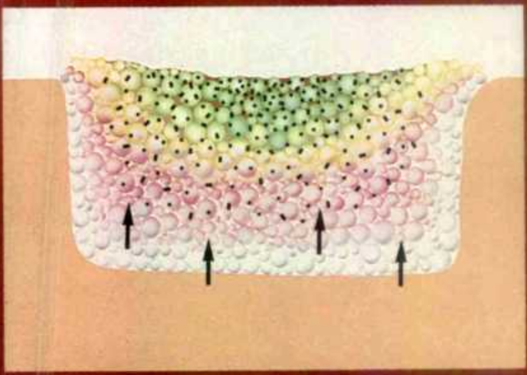
**Where** On all secreting wounds such as leg ulcers, pressure sores, post-surgical infections, Debrisan actively speeds healing.



**When** As soon as such wounds present, apply Debrisan and renew as necessary. 'The quality as well as the rapidity of wound healing has been impressive."[1]

**How** By capillary action Debrisan continuously removes wound exudate, with bacteria and fibrinogen drawn up into the top layer. Inflammatory mediators are removed and painful oedema then subsides. Local blood flow should improve and the wound base is rendered clean and granulating.



# ebrisan

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## Unique fast-acting wound therapy

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[1] Thorne N. (1979) Brit. J. clin. Pract. **33**, 21

3. When deep infected wounds are treated, care must be taken to wash Debrisan from the depths of the wound.
4. No side-effects have been reported.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Precautions:

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2. When exudate has been markedly removed by Debrisan alternative treatment should be substituted.
3. In order to avoid cross-infection it is recommended that the contents of a castor be confined to the treatment of a single patient for one day.

**Pharmaceutical precautions** Keep in a dry place in well-closed containers.

**Package quantities** Sachets of 4 g in boxes of seven. Basic NHS price £1.60 each. Castors of 60 g.

**Legal category** POM. **PL** 0009/0021

Debrisan (regd) dextranomer is a product of Pharmacia (Great Britain) Ltd, Prince Regent Road, Hounslow, Middlesex TW3 1NE. Telephone: 01-572 7321

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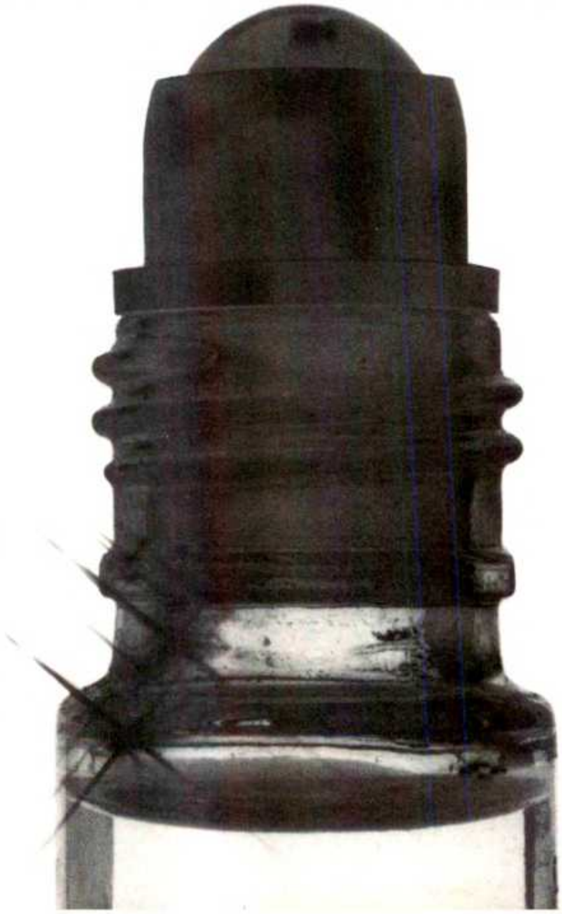

**Prescribing Information**

PRESENTATION:
Colourless evaporative solution containing 20% w/v aluminium chloride hexahydrate.

INDICATION:
For the topical treatment of hyperhidrosis specifically involving the axillae, hands or feet.

DIRECTIONS FOR USE:
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

PRECAUTIONS:
Restrict application to affected sites only. Keep away from the eyes.

PACKAGE QUANTITY AND BASIC NHS PRICE:
10ml glass roll-on bottle £2.15.

REFERENCE:
British Medical Journal, 1978, **2**, 84.

PL 0173/0030

*Anhydrol is a registered trade mark*

*British Journal of Dermatology* (1980) **102,** 629.

***Clinical and Laboratory Investigations***

# Flow cytometry as a tool for the study of cell kinetics in epidermis

## INVESTIGATIONS ON NORMAL EPIDERMIS

F.W.BAUER, N.H.C.M.N.CROMBAG, R.M.DE GROOD AND G.J.DE JONGH

Department of Dermatology, University of Nijmegen, Nijmegen, The Netherlands

Accepted for publication 26 September 1979

### SUMMARY

Flow cytometric measurements of the DNA content were performed on a large number of skin biopsies by an automated technique. Expressed as a percentage of all viable cells in the epidermis, the figures for cells in S-phase averaged 1·8% and for $G_2M$ 0·9%. No significant differences due to sex were found. Concomitantly with age the ratio $S/G_2M$ (representing the duration of S to the duration of $G_2M$) increased. Also seasonal effects were clear, showing higher values for S and $G_2M$ in June compared to November and December. Lastly we found small differences dependent on body-site, the ratio $S/G_2M$ being greater in legs than in arms. The present status is discussed together with future lines of development.

The development and application of high speed flow systems for the automated analysis of cell populations have added a new dimension to the fields of biology, biomedical research and clinical medicine. Up to now these methods have mostly been used in cancer research; however, it is our feeling that in due course they will find their way into dermatology as well.

We have already published some preliminary observations using this technique (Bauer & de Grood, 1975, 1976), in which we showed that the applications of flow cytometry to epidermis make it possible to measure cell cycle parameters of keratinocytes extremely quickly on large numbers of cells. Since the sampling of skin is easy and not harmful, many biopsies can be obtained. The purpose of the present communication is to describe our methodology in greater detail, to discuss certain theoretical aspects and to present our data for normal skin. The investigation of cell kinetics in psoriatic skin before and during treatment will be described in a subsequent publication.

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## MATERIALS AND METHODS

### *General techniques*

*Preparation of single cell suspensions.* The preparation that we will describe here is similar to that published earlier with minor modifications (Bauer & de Grood, 1976). Epidermal samples were obtained from healthy volunteers without skin complaints. The time of sampling was between 9.00 and 10.00 a.m. in order to exclude a possible diurnal rhythm.

Very superficially cut biopsies are obtained by the following procedure: a 6 mm punched hole in a metal guard allows the skin to protrude through it. A biopsy of about 3 mm in diameter and a thickness of about 0·5 mm can be obtained with a razor blade. The skin is incubated for 20 min at 37°C in a few drops of 0·15 M phosphate buffer, pH 7·8 ($Na_2HPO_4$, $NaH_2PO_4$) containing 1% trypsin (Difco 1:250) and 0·3% dithioerythritol (Sigma). After the incubation the intact specimens are transferred to a test tube with 0·5 ml 0·15 M phosphate buffer pH 7·8 containing propidium iodide (10 mg/l; the staining solution). After 10 s ultrasonic vibration at 70 W (Sonifier B-12, Branson Sonic Power Company), the epidermis detaches from the dermis and the epidermal cells dissociate. The stratum corneum and the dermis remain intact with this procedure, so that no admixture of dermal cells take place.

To the suspension is added 5 ml staining solution, two drops of DMSO and 0·1 ml RNA'ase (10 mg/ml; Sigma, 76 Kunitz units/mg). After filtration through gauze (mesh approx. 100 $\mu$m, supplied by Phywé, BRD) and a staining period of 15 min the cell suspension is ready for measurement. About 200,000–300,000 cells can be obtained per biopsy.

*Apparatus.* The apparatus we use is an impulse cytophotometer ICP 11 (Phywé, BRD) equipped with a sheath flow cell. The ICP 11 is connected with a calculator (Hewlett–Packard 9810A) by means of an interface (developed by our electronic department). The data are presented as a histogram by means of a plotter (Hewlett–Packard 9862A calculator plotter). The analysis is carried out by a specific program. More extended data manipulation can be performed via a link with a PDP 11/45 computer by means of a tape puncher.

*Curve analysis.* The DNA frequency distribution is assumed to consist of three subpopulations of cells: 2c cells (cells in $G_1$, $G_0$ and differentiated cells), 4c cells (cells in $G_2M$) and 2-4c cells (cells in S) (Fig. 1). There are different mathematical models (e.g. Dean & Jett, 1974) to analyse DNA distributions. Baisch & Linden (1975) described a planimetrical method, elegant in its simplicity and low cost. The model we use is a modification of this. Assuming that the rate of synthesis through the S-phase is linear and that the cells are randomly distributed over the cell cycle, the distribution of the cells in S is approximated by a rectangle between the 2c maximum of the joint distribution and the double of the 2c maximum. The height of the rectangle is indicated by the plateau between 2c and 4c in the histogram. The percentages of S, 2c and 4c cells are obtained by integrating the appropriate areas of the histogram. A look at a representative histogram (Fig. 2) indicates that this assumption is reasonable.

The difference from the 'Baisch' model is that in the latter the rectangle is drawn between the 2c and the $G_2M$ maxima of the joint distribution. Table 1 shows how far the rectangular model fits to simulated curves based on Gaussian distributions. One can observe that the smaller the coefficient of variation (the width of Gaussian distributions), the better the rectangular model approximates the simulated curves. The majority of the DNA distributions from normal and pathological epidermis can be analysed this way. With the planimetrical model described by Baisch & Linden (1975), the

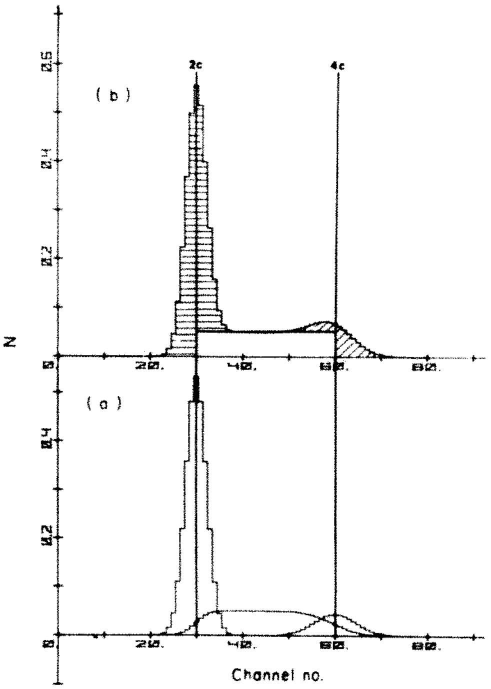
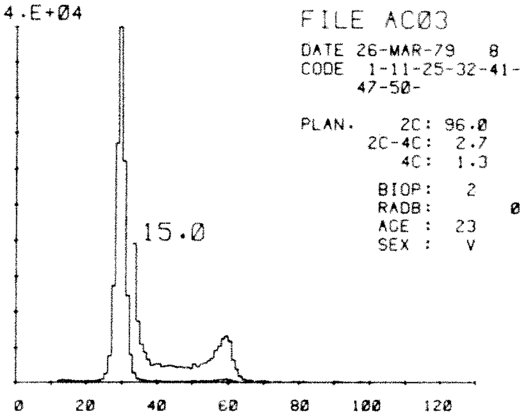


FIGURE 1. (a) Subdistributions of 2c, 2c-4c and 4c cells. (b) Joint distribution. Note the shift of the $G_2M$ maximum to the left.



FILE AC03
DATE 26-MAR-79 8
CODE 1-11-25-32-41-
47-50-
PLAN. 2C: 96.0
2C-4C: 2.7
4C: 1.3
BIOP: 2
RADB: 0
AGE : 23
SEX : V

FIGURE 2. Representative DNA distribution of the normal epidermis. Total cell number: 128,956.

fraction of cells in S is underestimated while $G_2M$ is overestimated due to the fact that the maximum of $G_2M$ shifts to the left through the underlying S cells.

*Statistical analysis.* Statistical analysis was done with S.P.S.S. (Statistical Package for the Social Sciences) subprograms (Nie, 1975; U.R.C., 1979). We first created a system file under S.P.S.S. conventions. The metric variables were: 2c, S, $G_2M$ ( = 100-2c-S) and the ratio $S/G_2M$. Non-metric

TABLE I.

| | Simulated DNA distributions | | | Calculated values with the modified planimetrical model | | |
|---|---|---|---|---|---|---|
| Var. coeff. | $G_1$ | S | $G_2M$ | $G_1$ | S | $G_2M$ |
| 0·05 | 95·00 | 4·00 | 1·00 | 95·01 | 4·01 | 0·98 |
| 0·075 | 95·00 | 4·00 | 1·00 | 95·02 | 4·02 | 0·95 |
| 0·10 | 95·00 | 4·00 | 1·00 | 95·02 | 4·08 | 0·89 |
| 0·05 | 85·00 | 10·00 | 5·00 | 85·03 | 10·03 | 4·95 |
| 0·075 | 85·00 | 10·00 | 5·00 | 85·05 | 10·07 | 4·88 |
| 0·10 | 85·00 | 10·00 | 5·00 | 85·03 | 10·30 | 4·67 |
| 0·05 | 80·00 | 15·00 | 5·00 | 80·04 | 15·04 | 4·92 |
| 0·075 | 80·00 | 15·00 | 5·00 | 80·08 | 15·09 | 4·82 |
| 0·10 | 80·00 | 15·00 | 5·00 | 80·09 | 15·32 | 4·59 |

categorical variables were: disease, age, season, sex, body site. This system file was then stored on on-line disc and used for all subsequent analyses.

For the purpose of the present investigation we selected the subfile of healthy controls, i.e. anyone with variable 'disease' = 0. Table 2 is the partial output of program 'condescriptive'; means and standard deviations are quoted here. Multiple regression was used to investigate the influence of age (Table 4). We used program 'anova' for the analysis of variance (Tables 3, 5–7). The selected output included frequencies (n), means and the significance tested at the 5% level (+ significant, − not significant). We opted for one main effect and no covariate throughout.

### *Pitfalls*

*Coincidence.* All particles are contained in a watery suspension and pass the detector area at a more or less constant velocity. If the space between two particles is big enough, they will be recorded as single events; if the space is small, the detector cannot separate the two particles, with the result that one particle with increased size will be recorded. In other words, if two $G_1$ cells follow too closely after each other, one particle with an S-value will be recorded and in the extreme case a 4c value. In slowly proliferating systems with low S-values coincidence becomes a source of serious error.

Figure 3 contains calibration curves from chicken erythrocytes, leukocytes in peripheral blood and for skin samples measured at different pulse rates. The intercept on the Y-axis is the real percentage of cells with 2c-4c values. The slope of the line varied a little from calibration to calibration, possibly due to variations in the flow system, resulting in changes in the signal width, for coincidence also depends on signal width. Calibration curves for human samples cannot be made routinely since they demand rather large quantities of material. Therefore we prefer a correction factor, derived from the calibration curve of peripheral blood or chicken erythrocytes. The correction we use is:

$$\text{Corrected } \%S = \text{measured } \%S - 0{\cdot}3\% \times \frac{\text{pulse rate}}{100}$$

where pulse rate is in cells/s.

$$\text{Corrected } \%G_2M = \text{measured } \%G_2M - 0{\cdot}12\% \times \frac{\text{pulse rate}}{100}$$

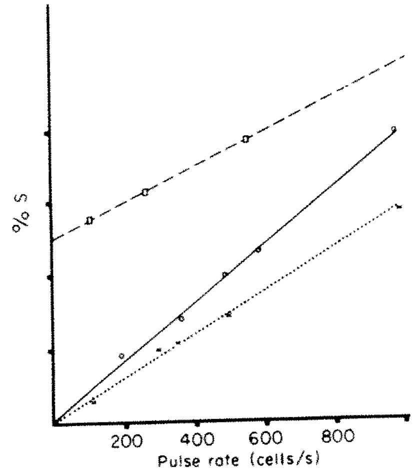


FIGURE 3. The effect of pulse rate on the percentage of signals recorded with values between 2c-4c (%S) (□ - - - - - - □) human skin; (○———○) peripheral leukocytes; (× - - - - - - ×) chicken erythrocytes.

Where indicated, the mean percentages of cells in S and $G_2M$ are corrected for coincidence. For a full discussion of coincidence problems (and an electronic solution based on the analysis of the width of the signal) see Göhde, Schumann & Früh (1976).

*Clumping*. Since the apparatus cannot distinguish between a 4c cell and a clump of two 2c cells, sample preparation requires as high a proportion of single cells as possible; otherwise the percentage of $G_2M$ cells is overestimated.

In data formerly presented by us, using another preparative procedure, this is the case (Bauer & de Grood, 1975). Fortunately, we find that using the trypsinization procedure described above the apparent 6c DNA (and hence the correction required) is in general negligible.

*'Odd' histograms*. Excessive breakdown of DNA will result in a large fraction of cells with less than 2c DNA. The histogram cannot in this case be analysed with the present mathematical procedure.

The same is true for histograms in which there is a block in early S or a synchronization wave in S, since the model requires a uniform distribution of the cells within the S-phase. These histograms occur in psoriatic skin during treatment, but are very rare in normal skin. Although these histograms contain a great deal of information, our normal analytical procedure is not appropriate.

### *Theoretical considerations*

With cytometry one measures DNA content of the cells and not DNA synthesis as is done with autoradiography. Most probably, however, a cell in normal skin that contains between 2c and 4c DNA will be a cell synthesizing DNA. Further, the %S and $G_2M$ are expressed as the percentage of all nucleated cells and not exclusively of cycling cells.

If we look at the percentage of cells which contain a DNA level between 2n and 4n (%S) the following equations hold true:

$$\%S = \frac{N_s}{N_{tot}} \times 100 \tag{1}$$

Related to cycling cells

$$\%S' = \frac{N_s}{N_{cycl.}} \times 100 \qquad (2)$$

and from (1) and (2):

$$\%S' = \frac{N_{tot}}{N_{cycl.}} \times \%S \qquad (3)$$

In this formula $N_{cycl.}/N_{tot}$ stands for the growth fraction*. The relation between turnover time of the viable epidermis (the time required for the production of a number of cells equal to the size of the compartment) and the cell cycle time is

$$T_{turn.ep.} = \frac{N_{tot}}{N_{cycl.}} \times T_c \qquad (4)$$

or

$$T_c = G.F. \times T_{turn.ep}$$

However,

$$\frac{T_c}{T_s} = \frac{N_{cycl.}}{N_s} \qquad (5)$$

Substituting (5) and (1) in (4):

$$T_{turn.ep.} = \frac{T_s}{\%S} \times 100 \qquad (6)$$

So if one is able to determine $T_s$, $T_{turn.ep.}$ can be easily calculated by flow cytometric measurements

The symbols are defined as follows:

$N_{tot}$ = number of cells in the viable epidermis;
$N_s$ = number of cells between 2c and 4c DNA;
$N_{cycl.}$ = number of cycling cells;
%S = the cells with DNA values between 2c and 4c as a percentage of the total cell number, as measured flow cytometrically;
%S′ = the cells with DNA values between 2c and 4c as a percentage of the cycling population;
$T_s$ = duration of S-phase;
$T_c$ = cell cycle time;
$T_{turn\ ep.}$ = turnover time of the viable epidermis;
G.F. = growth fraction.

## RESULTS

The values for normal skin are presented in Table 2. At first glance the value for the ratio $S/G_2M$. seems to be too high. The mean ratio, however, is obtained by averaging the parameter $S/G_2M$ and not by dividing the mean value of S by the mean of $G_2M$.

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* Growth fraction is defined here as that part of the total nucleated cells that is really cycling, and not as the part of the basal cells that is cycling.

TABLE 2. Analysis of the DNA distribution in normal skin based on the planimetrical model (means ± standard deviation). (A) Without correction for coincidence; (B) corrected for coincidence (mean pulse-rate 250 cells/s)

| | Normal skin n = 137<br>2c | S | $G_2M$ | $S/G_2M$ |
|---|---|---|---|---|
| A | 96·2 ± 1·0 | 2·6 ± 0·8 | 1.2 ± 0·5 | 2·5 ± 1·1 |
| B | 97·4 | 1·8 | 0·9 | — |

*Influence of sex*

We found no significant sex effect in the DNA distribution (Table 3).

*Influence of age*

Table 4 presents the influence of age in the measured parameters. In order to exclude variance due to season (see below), only measurements which were done in June were selected for analysis. Care was taken that there was a uniform site distribution in the age groups.

The percentage of 2c cells increases with age. The percentage of cells in S remains constant, whereas the percentage of $G_2M$ cells steadily drops. The ratio $S/G_2M$ also increases significantly.

*Influence of season*

Table 5 presents DNA distributions for skin biopsies in June, versus November and December. The differences in values for 2c, S and $G_2M$ are significant at the 5% level. The ratio $S/G_2M$ does not change with season.

TABLE 3. The influence of sex on the mean DNA distribution of normal epidermis

| | Male (n = 99) | Female (n = 38) | Significant at the 5% level |
|---|---|---|---|
| 2c | 96·3 | 96·1 | — |
| S | 2·6 | 2·6 | — |
| $G_2M$ | 1·2 | 1·3 | — |
| $S/G_2M$ | 2·5 | 2·4 | — |

TABLE 4. The influence of age

| | Increase/year (%) | Intercept Y-axis (age = 0) | Significant at the 5% level |
|---|---|---|---|
| 2c | 0·014 | 95·2 | + |
| S | — | 2·9 | — |
| $G_2M$ | −0·011 | 1·8 | + |
| $S/G_2M$ | 0·02 | 1·7 | + |

TABLE 5. Influence of season on the mean DNA distribution of normal skin

| | June (n = 60) | Nov/Dec (n = 77) | Significant at the 5% level |
|---|---|---|---|
| 2c | 95·9 | 96·5 | + |
| S | 2·7 | 2·4 | + |
| $G_2M$ | 1·3 | 1·1 | + |
| $S/G_2M$ | 2·4 | 2·6 | − |

### *Influence of body site*

Table 6 summarizes the figures for DNA distributions at three different body sites, Again, to eliminate seasonal influences only the data obtained in June were selected for analysis. For the same reason it was checked that there was a uniform age distribution through the groups.

We found significant differences as to the percentage of 2c, $G_2M$ cells and the ratio $S/G_2M$, whereas the percentage of S remained constant. Table 7 shows that these differences are caused mainly by differences between arms and legs.

TABLE 6. Influence of body site on the mean DNA distribution of normal epidermis (period June)

| | Arm (n = 15) | Back (n = 8) | Leg (n = 12) | Significant at the 5% level |
|---|---|---|---|---|
| 2c | 95·2 | 95·7 | 96·2 | + |
| S | 3·1 | 3·0 | 2·7 | − |
| $G_2M$ | 1·7 | 1·3 | 1·1 | + |
| $S/G_2M$ | 1·8 | 2·6 | 2·9 | + |

TABLE 7. Significance of differences in body site by using simple t-test

| | Arm–leg | Arm–back | Back–leg |
|---|---|---|---|
| 2c | + | − | − |
| S | − | − | − |
| $G_2M$ | + | + | − |
| $S/G_2M$ | + | + | − |

## DISCUSSION

Reports on cytophotometrical studies from normal or pathological human epidermis are rare in the literature. Grove, Anderton & Smith (1976) performed static cytophotometric studies on normal and psoriatic skin. In this study the DNA content was determined with a cytophotometer that scans Feulgen stained skin sections on a slide. This procedure is far slower than flow cytometric methods, and also the coefficient of variation which influences the analysis of the DNA distributions is far greater. For inherent difficulties of this method, e.g. partly cut nuclei, see Würthner *et al.* (1972). Nevertheless these authors were able to show differences in DNA distributions between normal epidermis and psoriatic epidermis. Haag, Tschahargane & Ehemann (1975) studied DNA content in single nuclei of human epidermis where the problem of partly cut nuclei was solved. No detailed analysis of the DNA distribution was made. Moreover analysis was confined to 500 cells. Flow cytometrical studies therefore are more promising for this kind of study.

The flow cytometrical measurements performed in this study were done on epithelial cell suspensions of whole viable epidermis. In this study we have assumed that we were dealing only with keratinocytes. This is of course not true. Other cell types are present as well but in relatively small numbers, so that even if they have very different kinetics their influence on the figure should be negligible.

Cell cycle times and turnover times cannot be determined by the present method because we have performed static measurements which give us at best relative cell cycle times. An estimation, however, could be made if we take values for $T_s$ from the literature. In steady state conditions:

$$T_{\text{turn.ep.}} = \frac{T_s}{\%S} \times 100$$

If we assume that $T_s = 8$ h (Gelfant, 1976) then

$$T_{\text{turn.ep.}} = \frac{8}{1{\cdot}8} \times 100 = 444 \text{ h} = 18{\cdot}5 \text{ days.}$$

This value fits rather well with turnover times calculated from studies with labelled amino acids (Rothberg, Crounse & Lee, 1961). The cell cycle time would become

$$T_c = \text{G.F.} \times T_{\text{turn.ep.}} = \frac{1}{3{\cdot}2}\,(\text{Halprin, 1972}) \times 18{\cdot}5 = 5{\cdot}8 \text{ days} = 139 \text{ h.}$$

Of course these are crude estimations.

The figures for S and $G_2M$ are lower than those previously reported by us (Bauer & de Grood, 1975) mainly since the methodological and the analytical procedures have been optimized.

For the statistical studies we have not tried to make corrections for the coincidence as on the average its contribution to the %S and $G_2M$ is not likely to be different in the groups that we are comparing.

Table 4 shows that there is an age dependency in some of the parameters that we are measuring. The interpretation, however, is fairly difficult. Increase in the percentage of 2c cells can originate from many factors, e.g. increase of $G_1$ or increase in the growth fraction. The fact, however, that the ratio $S/G_2M$ increases with age means that the relative duration of S to $G_2M$ gets longer, i.e. it reflects real changes in the cell cycle.

Table 5 shows that there are seasonal variations (or strictly speaking, differences between two specific times of the year) in the percentage of cells in S and in $G_2M$. However, the ratio $S/G_2M$ does not change. At the moment we can also only speculate about the meaning of the differences since we have too little data. Possible explanations, for example, are that the growth fraction in summer is higher than in winter (which would be reflected by a faster turnover), that the durations of both S and $G_2M$ are prolonged in summer, or that the durations have stayed the same whereas $G_2$ is shortened.

We have also demonstrated small site-dependent differences. Particularly if we compare arm to leg we find a statistically significant difference in the 2c value (higher in legs) and $G_2M$ (lower in legs). Also the ratio $S/G_2M$ is higher in legs, indicating differences in the cell cycle phases at different parts of the body.

In conclusion, it seems useful to comment upon the present status of flow cytometry and its future prospects in dermatological research. Flow cytometric determinations of the DNA content of keratinocytes can be done very rapidly on large numbers of cells from a relatively small piece of skin (±3 mm ø). The sampling of skin is elegant, the cell loss very low. Due to the minimal discomfort for the patient sampling can be done often. Fifty pieces from one patient is no exception in our studies. In principle it is therefore possible to follow DNA distributions during therapy.

The flow cytometric method as we use it now has certain limitations. Technical problems (e.g. coincidence) have largely been resolved, although doubtless some further improvements could still be made using our present apparatus. A major disadvantage of the method is the lack of histological control. In theory one can obtain similar DNA distributions from two skin samples that are quite different histologically, for example in thickness. In part, this could be overcome by making total cell counts under a standardized surface area.

Instruments are now commercially available that can measure two or more fluorescence parameters simultaneously in one cell or a light scatter parameter (that gives information about cell size or shape) and a fluorescent parameter. So if we succeed in finding markers which are specific for the various cell types then we will also be able to discriminate between epidermal populations that cannot be differentiated on DNA alone. Based on histological evidence, candidates for that second parameter in skin seem to be either RNA or protein. Further, instrumental developments have led to sorting machines. Cells of interest (characterized by certain parameters) can be sorted out from the total suspension so that afterwards cytological control is possible. At the moment, however, the instrumental developments precede the biological preparative techniques.

In this study we have made static measurements that at best will inform us about relative cell cycle times. In that respect they resemble the information one can obtain from autoradiographic studies after pulse labelling with $^3$H thymidine. With certain autoradiographic techniques one can derive absolute cell cycle times, namely by double labelling with $^3$H thymidine and $^{14}$C thymidine or by making PLM curves (percentage labelled mitosis curves). Other workers have calculated absolute cell cycle times by the use of stathmokinetic methods, for example the determination of the mitotic rate by colchicine blocking. For an excellent review of these techniques see Aherne, Camplejohn & Wright (1977). A way to obtain $T_s$ from flow cytometric measurements was recently described by Gray (1976). Cells in S were labelled with $^3$H thymidine and cells in mid S-phase (3c) were sorted out electronically. By liquid scintillation counting they measured the variation in radioactivity in mid S. From this kind of study they were able to obtain $T_s$ and $T_c$.

Absolute cell cycle times can in principle also be calculated from flow cytometric measurements by analysing the changing DNA distributions due to drug interactions.

Recently, progress has been made in the mathematical description of the disturbances in the cell cycle due to drug action (Fried *et al.*, 1976; Gray, 1976; Kim, Wheeler & Perry, 1978). This description will provide the absolute cell cycle times at the moment of biopsy and may be extrapolated to calculate future patterns of DNA distribution.

So, in conclusion, we feel that flow cytometric studies are already valuable for studying cell kinetics in spite of the shortcomings. Many of the latter will in the future be overcome by the combination of new biological staining techniques with up-to-date machines. Ultimately they may enable us to rationalize therapy in proliferative skin diseases.

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# Maturation of normal human epidermis without an ordered structure

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## SUMMARY

Whole mounts of epidermis were prepared from hamster and mouse ears and from three sites on six normal human volunteers. Corneocyte columns were sought visually with the light microscope and statistically with cluster analysis applied to the surface distribution of superficial nucleated keratinocytes. In fifteen consecutive fields of hamster and mouse epidermis, both cell columns and clustering of nuclei were detected. In eighteen consecutive biopsies from the forearm, back and thigh of six normal adult men, stacking was never seen and clustering could not be demonstrated statistically. Corneocyte columns do occur regularly in some mammalian species and they have been reported to occur in human skin, but such columns are not common in these sites from normal human skin.

The stratum corneum of mammalian epidermis consists of multiple layers of flattened, anucleate corneocytes. At the skin surface these cells exfoliate, individually and in small aggregates, to the environment. This loss by exfoliation is balanced by proliferation and maturation of underlying nucleated keratinocytes (Bergstresser & Taylor, 1977). In cutaneous disorders such as psoriasis (Weinstein & Van Scott, 1965), or in response to environmental stresses such as tape stripping (Pinkus, 1951), retinoic acid application (Braun-Falco & Christophers, 1969) or ultraviolet light irradiation (Epstein, Fukuyama & Fye, 1970) the rate of proliferation, maturation and exfoliation is greatly accelerated.

It has been demonstrated in certain mammalian species that both the compressed, keratinizing cells of the stratum spinosum and the flat corneocytes of the stratum corneum tend to be stacked in an ordered structure (MacKenzie, 1969, 1970, 1972; Christophers, 1971a; Menton & Eisen, 1971a). This ordered structure has been visualized consistently and most easily in the ear skin of the hamster, mouse, rat, guinea-pig, and rabbit (MacKenzie, 1969; Christophers, 1971a; Menton & Eisen, 1971a). It has also been reported for monkey thorax (MacKenzie, 1972) and for human thorax (Menton & Eisen, 1971a; MacKenzie, 1975). An important aspect of these studies is the associated observation

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that stacking begins in the underlying stratum spinosum where columns of nucleated spinous cells may be observed (Christophers, 1971a).

A major question is whether stacking is a fundamental or obligate biological property of mammalian epidermis. This question arises from the implication that stacking is the normal and common state of all mammalian skin. If it is, as has been proposed, then one must ask why it was observed only recently in human skin. Stacking of human stratum corneum could be missed easily since electron microscopy and special techniques are required for its demonstration, but the antecedent stacking of spinous layer keratinocytes in lower mammals is quite characteristic and should have been noticed in human skin. An alternative conclusion is that stacking is in fact not common in normal human skin.

Epidermal structure is easily visualized in three dimensional whole mounts of epidermis, although this visualization is difficult to translate either into two dimensional figures or into data. We have developed a histological and statistical technique to assess the distribution of superficial epidermal nuclei in whole mounts of epidermis. It was used to search for cell columns in epidermal specimens from two laboratory animals known to exhibit columns and in human epidermis from six normal subjects.

## MATERIALS AND METHODS

### *Epidermal whole mounts*

Skin biopsies were taken from six adult male patients who were selected on the basis of a skin disorder limited to the face or feet. Each gave informed consent and was biopsied at three normal appearing sites: (a) volar forearm, (b) upper back, and (c) upper lateral thigh. These patients, whose ages ranged from 46 to 72 years, were used in a previous study in which epidermal cell volumes and surface keratinocyte densities were determined (Bergstresser, Pariser & Taylor, 1978). Specimens were prepared as described in that study. Briefly, freehand skin biopsies were incubated for 30–60 min at 37°C in 1 mg/ml crude bacterial collagenase (Sigma Chemical Co., St Louis). After mechanical separation, the epidermis was fixed, stained with haematoxylin, dehydrated and mounted, basal cell surface up, on microscope slides.

### *Laboratory animals*

One adult pigmented (CB) hamster and four adult albino (AJAX) mice were killed with ether anaesthesia. Depilated ear skin was separated by blunt dissection from the underlying cartilage and epidermal separation was performed either with collagenase as above or with incubation in 2 N NaBr at 37°C for 120 min. After separation, epidermal whole mounts were prepared as above.

Epidermal specimens were examined at all powers with transmission light microscopy. The stratum corneum became relatively transparent, although, in the small animal skin, cell column borders were visible. Cell nuclei were easily seen.

### *Surface distribution of cell nuclei*

At oil immersion power (× 1000) the entire mount was examined with a calibrated 10 by 10 division grid (American Optical Co., #408A) in the eyepiece. At this power the depth of focus was about 3$\mu$m, and cells outside this limit were imperceptible. Nuclei at or near the basal cell layer appeared small and ellipsoidal with the long axis in the vertical dimension. As the plane of focus passed through the specimen, the more superficial nuclei occupied a greater surface area but their thickness (depth) became much smaller. For this reason it was easy to contrast superficial spinous cells with the deeper cells. Within the grid each superficial cell was identified and its grid location was determined. In some samples, the location of all nuclei was determined so that differences between superficial and deep nuclei could be ascertained.

*Data analysis*

Superficial nuclei visualized within the entire grid numbered between 50 and 150 with a mean density of 0·5–1·5 cells per small square. When nuclei formed stacked columns, a disproportionate number were clustered in relatively few squares. Since the mean number of nuclei was relatively small, the distribution of random events approximated the Poisson distribution (Hays & Winkler, 1970). To assess whether the distribution across the grid was random or clustered, the frequency distribution of nuclei within small squares was determined and compared with the distribution predicted by the Poisson equation (Hays & Winkler, 1970):

$$P\mathrm{r} = \frac{\mathrm{e}^{\mathrm{A}}\mathrm{A}^{\mathrm{r}}}{\mathrm{r}!}$$

where $P$ = probability that a square will contain r nuclei;
A = mean number of nuclei per square for the entire grid;
e = 2·718;
! = factorial.

The chi square test was used to determine whether observed distributions were non-random. An even distribution of nuclei across a grid appeared non-random as well. Consequently, both grid distributions and frequency distributions were examined together. If nuclei were clustered, squares without nuclei increased in number, while if nuclei were evenly distributed, squares without nuclei decreased in number.

To determine whether a stimulus known to obliterate cell columns would alter the distribution observed with this technique, several mouse ears were treated once daily for 4 days with 0·05% retinoic acid in a cream base (Retin-A, Johnson and Johnson, New Brunswick, NJ).

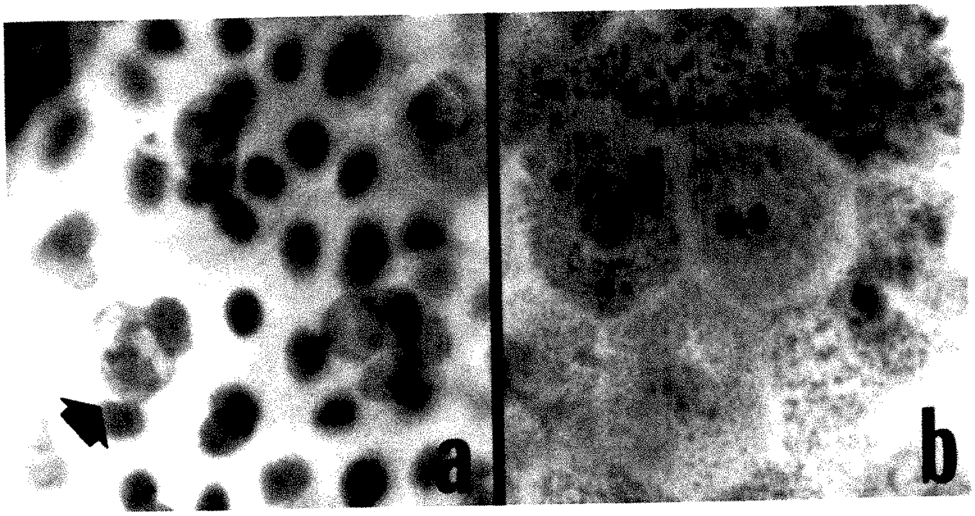

FIGURE 1. (a) Whole mount of interfollicular mouse epidermis which is stained with haematoxylin. Three superimposed columns of superficial, flattened nuclei are visible (arrow). (b) Hexagonal cell columns as seen in the stratum granulosum of the same epidermal whole mount. Each occurs above a column of epidermal nuclei.

## RESULTS

### *Laboratory animals*

Large portions of interfollicular mouse and hamster epidermis exhibited superficial cell nuclei stacked vertically above one another (Fig. 1a). As the plane of focus passed into the granular layer and the stratum corneum, the characteristic hexagonal cell column also became apparent (Fig. 1b). Fifteen areas were enumerated in six specimens of hamster, and mouse ear. A typical assessment is illustrated in Fig. 2, and the expected and observed frequencies are listed in Table 1. In each assessment, the *P* value for the chi square test was less than 0·05 and most often it was 0·001 or less (Table 2).

In mouse ear skin pretreated for 4 days with a daily application of 0·05% retinoic acid, cells were enumerated and located. Stacking was not observed visually. In two blindly selected sites, the distribution of superficial cells was non-random but in the direction of an even distribution (Table 3).

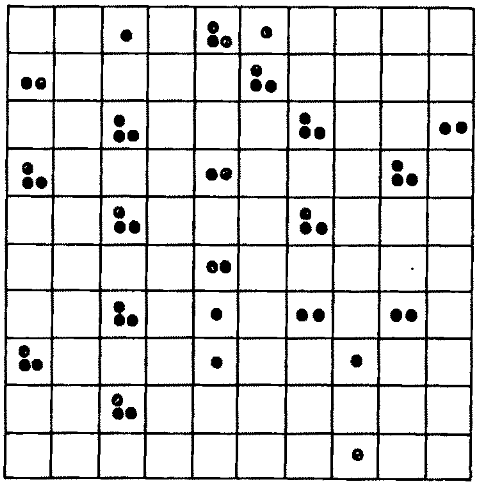

FIGURE 2. Observed distribution of superficial nuclei in a whole mount of mouse epidermis. A disproportionate number of squares contain three nuclei (Table 1).

TABLE 1. Frequency distribution of superficial nuclei in a whole mount of mouse epidermis (Fig. 2). Observed frequencies in the 100 square grid are contrasted with expected frequencies derived from the Poisson distribution

| Nuclei per square | Frequency | |
|---|---|---|
| | Observed | Expected |
| None | 77 | 60 |
| 1 | 6 | 31 |
| 2 | 6 | 8 |
| 3 | 11 | 1 |

$P < 0{\cdot}001$ (chi$^2$).

TABLE 2. $P$ values derived from the chi$^2$ test of significance for observed and expected frequencies of superficial epidermal nuclei. In all mouse and hamster specimens there was a significant non-random distribution with a clustering of nuclei

| Mouse | | Hamster | |
|---|---|---|---|
| Specimen | $P$ value | Specimen | $P$ value |
| 1 | <0·02 | 1 | 0·04 |
| 2 | <0·004 | 2 | 0·005 |
| 3 | 0·001 | 3 | 0·002 |
| 4–11 | <0·001 | 4 | <0·001 |

TABLE 3. Frequency distribution of superficial nuclei in a whole mount of mouse ear epidermis treated daily ×4 with topical retinoic acid. The Poisson distribution is skewed towards a more even one

| Nuclei per square | Frequency | |
|---|---|---|
| | Observed | Expected |
| None | 6 | 19 |
| 1 | 37 | 31 |
| 2 | 41 | 26 |
| 3 | 14 | 15 |
| 4 (or more) | 2 | 9 |

$P = 0{\cdot}007$ (chi$^2$).

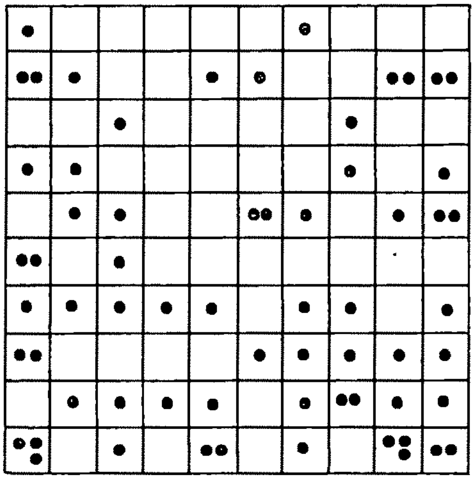

FIGURE 3. Observed distribution of superficial nuclei in a whole mount of human epidermis. The frequency distribution is random (Table 4).

Total epidermal cell populations were also determined in mouse ear skin from two treated and two untreated sites. They increased from 239 and 336 in the untreated sites to 686 and 505 in the treated sites. The mean surface densities of nucleated epidermal cells consequently increased from $3{\cdot}26 \times 10^4$ to $6{\cdot}19 \times 10^4/\text{mm}^2$.

### *Human skin*

In eighteen epidermal specimens from three sites (volar forearm, upper back, and upper lateral thigh) in six male subjects, clustering or stacking of superficial epidermal cells was never observed. In three blindly selected areas of each biopsy specimen, superficial cells were identified and assigned to the 100 square grid. A typical grid is illustrated in Fig. 3 ($P = 0 \pm 840$). In forty-seven of fifty-four fields, the observed distribution was not different from the expected Poisson distribution, as revealed by non-significant *P* values of between 0·1 and 1·00 (Table 4). In seven analysed fields the *P* value was less than 0·05. In four of these, nuclei were evenly distributed over the field; and in three, nuclei were absent from a large area and randomly distributed over the rest of the field. Clustering of nuclei was never observed visually and could not be demonstrated in the statistical analysis.

TABLE 4. *P* values derived from the chi$^2$ test of significance for observed and expected frequencies of superficial epidermal nuclei. No clustering was detected in fifty-four examinations of eighteen human biopsies

| Specimen | *P* value |
|---|---|
| 1–32 | 0·5–1·0 |
| 33–45 | 0·2–0·5 |
| 46–47 | 0·1–0·2 |
| 48–50 | <0·05* |
| 51–54 | <0·01* |

* Nuclei evenly distributed or absent from a large portion of the grid.

## DISCUSSION

We have examined haematoxylin-stained whole mounts of mouse, hamster and human epidermis. In the mouse and hamster ear, columns of haematoxylin-stained, superficial, nucleated keratinocytes were observed. With a superimposed optical grid the frequency distribution of nuclei was compared with that predicted by the Poisson distribution, and in fifteen consecutive fields of mouse and hamster ear epidermis, the superficial nuclei were clustered. Retinoic acid obliterated corneocyte columns within 4 days, and it doubled the surface density of epidermal cells ($3{\cdot}24 \times 10^4$ to $6{\cdot}19 \times 10^4/\text{mm}^2$). In contrast, stacking or clustering of superficial nuclei was never observed in eighteen consecutive biopsies of normal human skin from six subjects. Furthermore, in fifty-four consecutive and blindly chosen fields, clustering as judged with the statistical test was never detected.

Four groups of investigators (Menton & Eisen, 1971a; MacKenzie 1969, 1970, 1972; Christophers, 1971a; Potten & Allen, 1976) have observed an ordered epidermal structure to occur commonly in mammalian skin. Furthermore, the underlying nucleated portion of the epidermis exhibited superficial keratinocytes in a similar alignment, causing individual nuclei within superficial portions of the stratum spinosum to appear stacked as well. Data from human skin are less available. By electron microscopy Menton & Eisen (1971a) observed corneocyte columns in a biopsy from the mid-chest of an adult man, and MacKenzie (1975) observed it as well in abdominal skin biopsy treated by alkaline expansion. These are the only published instances of column formation in normal human skin, although Menton & Eisen (1971b) observed that corneocyte columns do occur in ichthyosis vulgaris. Columns were absent, however, in psoriasis, lamellar ichthyosis and lichenified skin. Since mitotic and proliferative rates are decreased in ichthyosis vulgaris (Frost & Weinstein, 1971; Frost, Weinstein & Van Scott, 1966; Frost & Van Scott, 1966), while they are increased in the other three disorders, stacking was observed only with decelerated proliferation.

The mechanism by which keratinocytes assume an ordered structure is not known. MacKenzie (1972) and Allen & Potten (1974) have observed that the distribution of epidermal Langerhans cells corresponds closely with individual cell columns. On the basis of this observation and the inverse relationship between epidermal proliferation and surface density of Langerhans cells, Potten & Allen (1976) have proposed that these dendritic cells exert physiological control over proliferation. Thus, columns are ordered by extrinsic forces from Langerhans cells. This interpretation is now in conflict with observations implicating Langerhans cells as pivotal in the acquisition of delayed contact hypersensitivity (Silberberg, Baer & Rosenthal, 1936; Stingl *et al.*, 1978). For one cell type of control two such diverse functions would be extraordinary.

A more plausible mechanism has been proposed by Menton (1976). He observed that cells which exhibit close stacking take the shape of a truncated tetrakaidecahedron, which is at a low thermodynamic energy state because of a minimized surface to volume ratio (Menton, 1976). Consequently, cell columns may arise from the minimization of surface areas of closely packed, semiliquid objects (Thompson, 1972; Allen & Potten, 1976). Stacking may occur only when there is sufficient time to allow cellular conformation to reach a low thermodynamic energy state. In ichthyosis vulgaris, a disorder of decreased proliferation, one observes an ordered structure. Furthermore, accelerated keratinocyte proliferation tends to obliterate stacking in those animals which ordinarily exhibit it (Christophers, 1971b). One may therefore hypothesize that stacking occurs, not from an inherent cellular structure, but on thermodynamic grounds alone.

Why columns do not occur in these skin sites from normal human skin has not been answered. This question is particularly intriguing since columns are reported to occur regularly in the monkey (*Macacque irus*) which is phylogenetically close to humans (MacKenzie & Linder, 1973). Perhaps the absence of hair or the desquamation of bathing accelerates keratinocyte proliferation and interrupts column formation. This interpretation is consistent with our observation with topical retinoic acid in the mouse and with the observation of guinea-pig skin after mechanical injury (Christophers, 1971b): that insults which accelerate proliferation may obliterate corneocyte column formation. Although these mechanisms are obviously not established, we may conclude that an ordered epidermal structure cannot be detected in multiple specimens of normal human skin.

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# Distribution of glycoproteins containing fucose in normal and psoriatic keratinocytes

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## SUMMARY

Electron microscope autoradiography has been used to compare the fucose-containing glycoproteins of normal and psoriatic epidermis. Normal keratinocytes and uninvolved psoriatic cells have most of their fucose on the plasma membrane, whereas involved psoriatic cells retain more within the cytoplasm. The fucose distribution in other epidermal cell types is also described.

Several studies have been made of the surfaces of epidermal cells in both normal and diseased skin (Mercer & Maibach, 1968; Hashimoto & Lever, 1970; Fritsch, Wolff & Honigsmann, 1975). Of particular interest to us was the finding of Orfanos, Schaumber-Lever & Mahrle (1973), based on the results of the periodic acid-silver methenamine (PASM) staining technique, that the surface of the keratinocytes in involved skin from psoriatic patients contained less carbohydrate material than that of keratinocytes from uninvolved psoriatic skin. In a further study in which they used alcian blue and lanthanum staining Orfanos, Mahrle & Runne (1976) showed disorganization of what they termed surface glycoconjugates.

Fucose is a terminal constituent of many carbohydrate chains that form part of the cell surface glycoproteins, and the measurement of the uptake and distribution of fucose in cells is a more precise technique than histological staining for assessing possible changes in components of the cell surface. ($^3$H)-L-Fucose has been widely used in studies of the surface glycoproteins of tumour cells (Buck, Glick & Warren, 1970; Warren, Fuhrer & Buck, 1973; Warren *et al.*, 1974), in studies on the synthesis and turnover of cell surface glycoproteins (Leblond & Bennet, 1974) and on the biosynthesis of glycoproteins in plasma membranes (Bennet & Leblond, 1977).

To gain information on the possible differences in the surface glycoproteins of normal epidermal cells compared with those of psoriatic epidermal cells we have measured the distribution of ($^3$H)-L-fucose within and on the surface of epidermal cells by electron microscopical autoradiography. We present our results in this paper.

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## MATERIALS AND METHODS

Specimens of involved and uninvolved psoriatic skin were obtained from volunteer patients. Wherever possible, a control sample of skin from a normal volunteer was taken at the same time and treated in the same way as the diseased skin. In addition, several normal skin samples obtained at plastic surgery were also processed.

The skin was trimmed so that it consisted mostly of epidermis with a supporting framework of dermis. Organ cultures were prepared by floating the skin, dermis down, on culture medium in a Bijou bottle. The medium consisted of Eagles minimum essential medium buffered with 1·0 M HEPES, pH 7·3, and with the addition of 10% fetal calf serum. ($^3$H)-L-Fucose (from the Radiochemical Centre, Amersham) in distilled water was added to a final concentration of 10 μci/ml. The bottles were gassed with a mixture of 10% carbon dioxide in air and incubated at 37°C. Incubations were carried out for 4, 8, 12, 24 and 48 h. At the end of the incubation period, the culture medium was removed, the skin washed in phosphate buffered saline, and then replaced with 3% glutaraldehyde in 0·2 M cacodylate buffer pH 7·4. Each skin sample was cut into four pieces and fixation was continued at 4°C for 3–5 h. Afterwards, specimens were washed in several changes of cacodylate buffer, postfixed in 2% aqueous osmium tetroxide, dehydrated in graded ethanols and embedded in Spurr resin. Survey sections 1 μm thick were stained with toluidine blue to ensure correct orientation before thin sections were cut. Ultrathin sections were cut with glass knives and collected on Formvar coated nickel grids. The grids and sections were carbon coated and then stained with uranyl acetate and lead citrate. The grids were mounted by their edges on a glass microscope slide using double sided sellotape and then coated with a monolayer of Ilford IL4 nuclear emulsion pulled on a nickel chrome wire loop. The coated grids were stored in light-tight boxes at 4°C for 16 days and then developed in D19b developer (Kodak) and fixed in Ilfofix (Ilford). All grids were checked for background level and any with an appreciable number of silver grains on the resin beyond the sections were discarded. Wherever possible, photographs were taken in basal, spinous and granular layers separately. Photographs were taken at ×3000 magnification and prints were made at ×7500. At least twenty photographs of each epidermal cell layer of each specimen were taken. The total number of silver grains, each representing a molecule of ($^3$H)-L-fucose, in each photograph was counted and separate counts were made of those that were membrane located and those that were intracytoplasmic. The number of cells per photograph was also counted and results were expressed as counts per cell ± s.e. mean and as percentage of grains which were plasma membrane associated. A silver grain was deemed to be membrane associated if it was within a circle of two silver grain diameters from the membrane. Each sample was counted by two people to reduce operator bias.

Although the epiboly was examined, counts in this area were not included as they were felt to be atypical. Melanocytes and Langerhans cells were considered separately as they showed a different labelling pattern to the keratinocytes.

## RESULTS

The results shown here comprise figures from four normal skin samples, four uninvolved psoriatics, and six involved psoriatics. Normal skin samples survived well under culture conditions. By 24 h epiboly had started and often by 48 h was complete around the underside of the dermis. The uninvolved psoriatic skin also survived well but the involved psoriatic was more unpredictable and more samples in this category died.

In all cases, the normal skin showed no abnormal features by light or electron microscopy. The uninvolved psoriatic skin was very similar to the normal, with only occasional slight thickening of the epidermis. The samples of involved psoriatic skin showed the light and electron microscopical changes typical of the disease. Desmosomes were small, some cells were oedematous, the epidermis was much thickened, there were reduced amounts of keratohyalin and the stratum corneum was parakeratotic. The basal lamina showed breaks and replication in some areas and this disruption had enabled inflammatory cells to invade the epidermis. Because of the reduced viability of the psoriatic skin, great care had to be exercised in selecting appropriate areas for doing grain counts, and only those fields with a full complement of living cells were used.

Counts of cell numbers showed that there was considerable variation in the number of cells per field (mean of 1·5 ± 3·9). Therefore results were expressed as a percentage of the total silver grains per cell, either localized in the plasma membrane or in the cell cytoplasm. The first time interval at which samples were examined was 4 h and silver grains were present on cells in the basal, spinous and granular layers of the epidermis. Cells in the granular layer had fewer silver grains than cells in the lower layers. After 12 h all three layers were labelled equally with silver grains. Silver grains were not present in the stratum corneum in any of the samples examined, either normal or psoriatic. Routinely all comparisons of skin samples were made after an incubation time with ($^3$H)-L-fucose of 24 h though in general the total number of silver grains per cell increased with time up to 48 h. The distribution pattern of grains between the plasma membrane and the cell cytoplasm for the keratinocytes of a particular sample of epidermis was constant and did not vary, irrespective of whether the cells were from the basal, spinous or granular layers.

The amount of incorporation of ($^3$H)-L-fucose varied considerably between different samples (Fig. 1) and a delay between obtaining the sample and setting up the culture system caused a reduction in incorporation but did not affect the distribution pattern of fucose. Samples of involved psoriatic epidermis were inconsistent in their incorporation of fucose relative to corresponding uninvolved

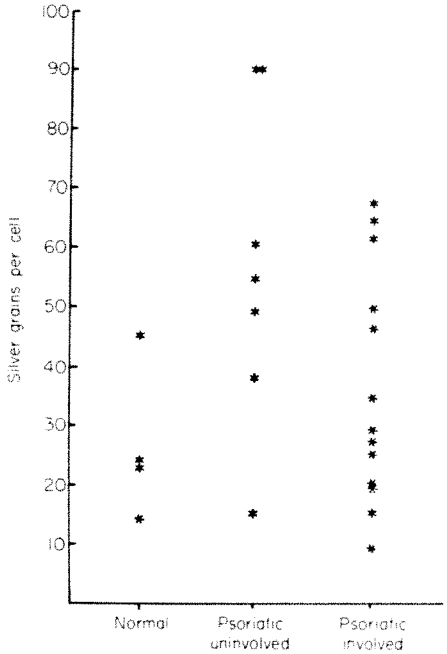

FIGURE 1. Number of silver grains per cell after 24 h incubation *in vitro*. Each point represents mean of at least fifty cells. The average s.e. mean is ± 10%.

samples, some being higher and others lower. However, there were consistent differences in the distribution patterns of silver grains in normal keratinocytes compared with those from uninvolved and involved psoriatic keratinocytes. In all normal samples most of the ($^3$H)-L-fucose was localized in the plasma membrane of the cell (Table 1), the remainder being present in the Golgi regions and in the cytoplasm. The uninvolved psoriatic cells consistently showed a smaller proportion of ($^3$H)-L-fucose in the plasma membrane and in the involved psoriatic cells the major proportion of the incorporated ($^3$H)-L-fucose remained within the cell. This pattern was not affected by cell size or degree of keratinization. The differences in distribution patterns are shown in Figs 2–4.

TABLE 1. Percentage of plasma membrane localized silver grains per cell

|  | Psoriatic |  |
|---|---|---|
| Normal | Uninvolved | Involved |
| n = 226<br>79·3 ± 8·9 | n = 284<br>62·1 ± 14·8 | n = 459<br>44·1 ± 9·2 |

n = number of cells.

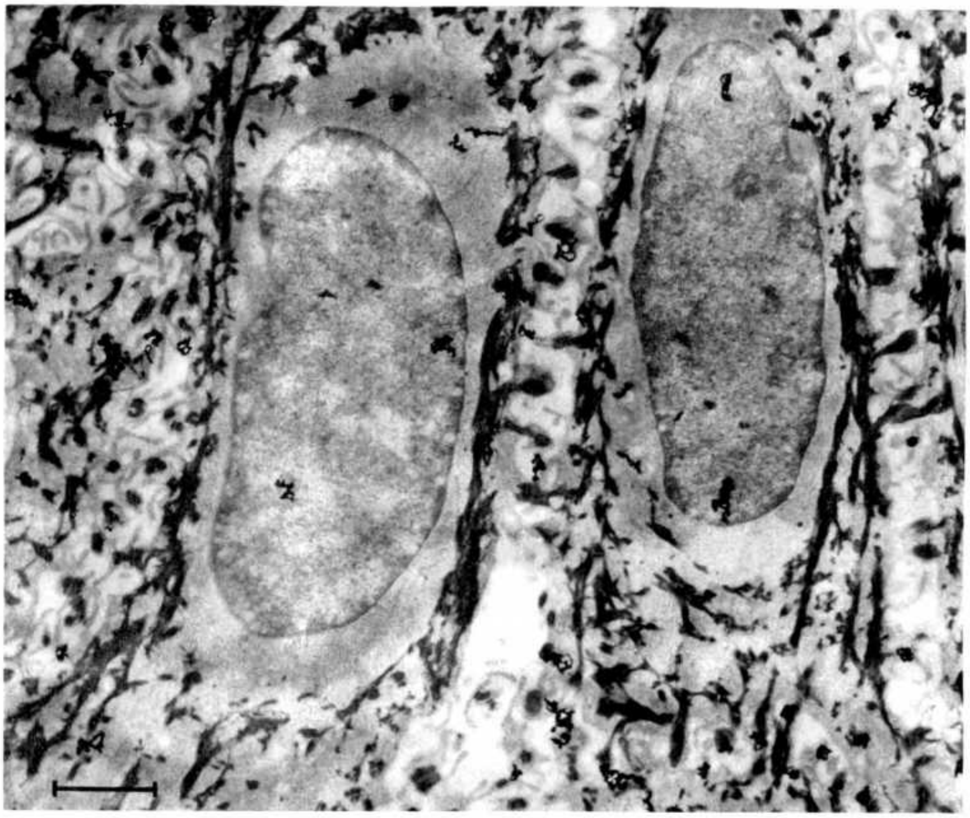

FIGURE 2. Autoradiograph of normal human skin after 24 h incubation. The majority of silver grains are on the plasma membrane or in the Golgi zone. Bar = 2 $\mu$m.

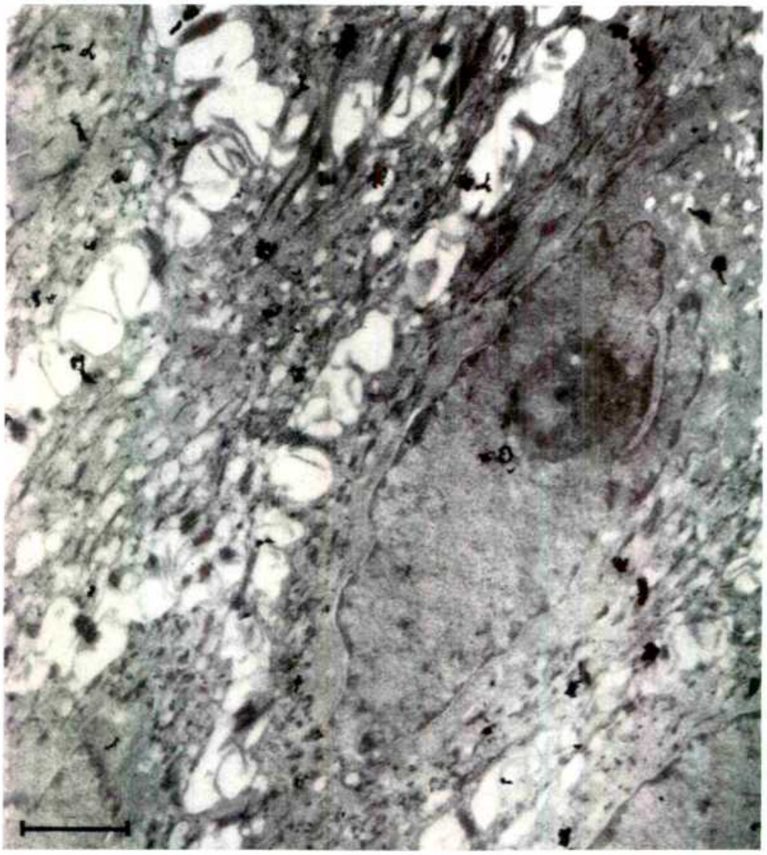

FIGURE 3. Autoradiograph of uninvolved psoriatic skin after 24 h incubation showing many silver grains on the plasma membrane. Bar = 2 μm.

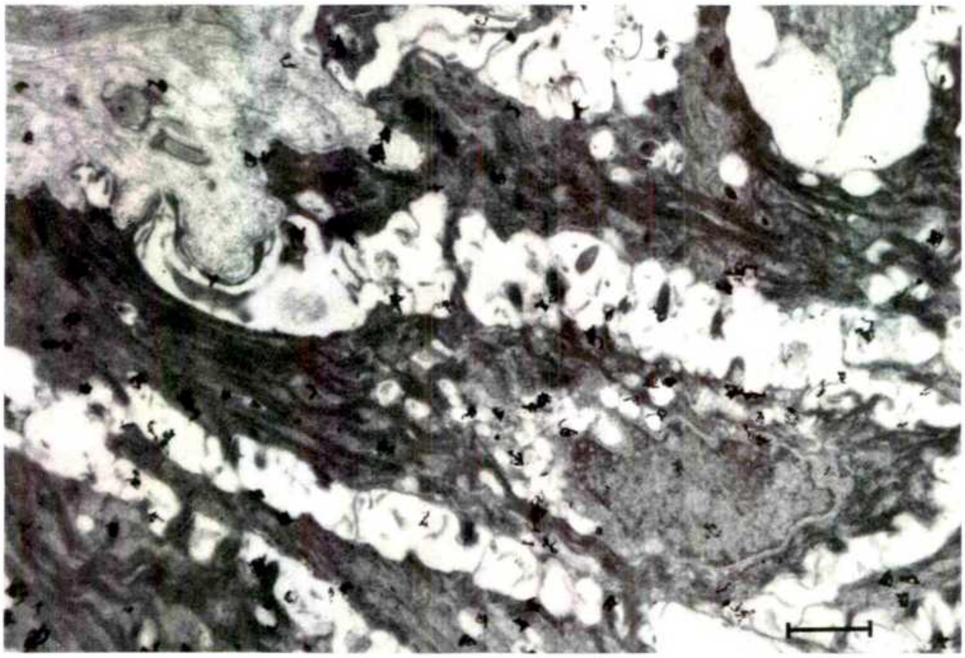

FIGURE 4. Autoradiograph of involved psoriatic skin showing a larger proportion of silver grains in the cytoplasm. Bar = 2 μm.

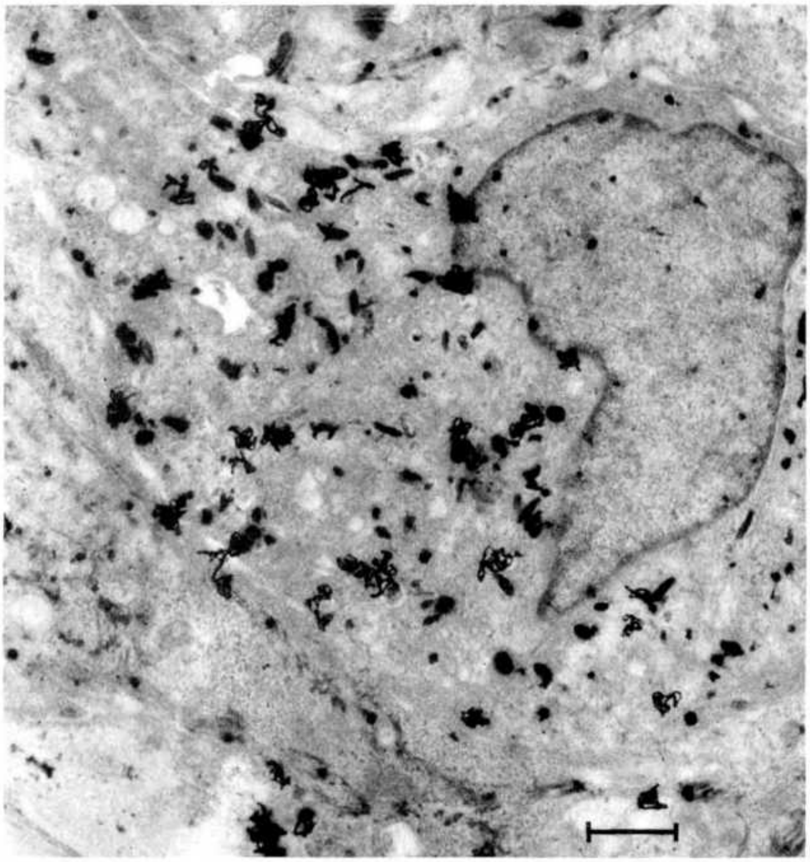

FIGURE 5. An autoradiograph of a melanocyte showing labelling associated with melanin granules and with the plasma membrane. Bar = 1 $\mu$m.

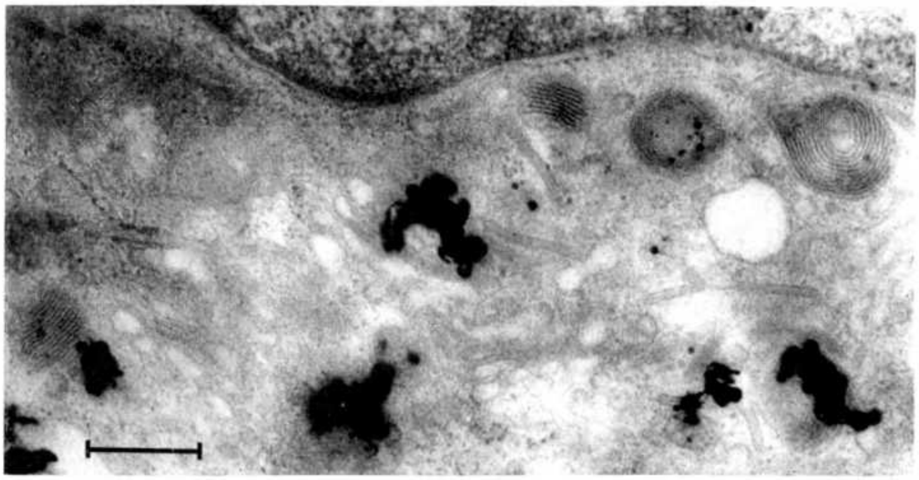

FIGURE 6. An autoradiograph of part of a Langerhans cell showing silver grains associated with the Langerhans granules. Bar = 0·5 $\mu$m.

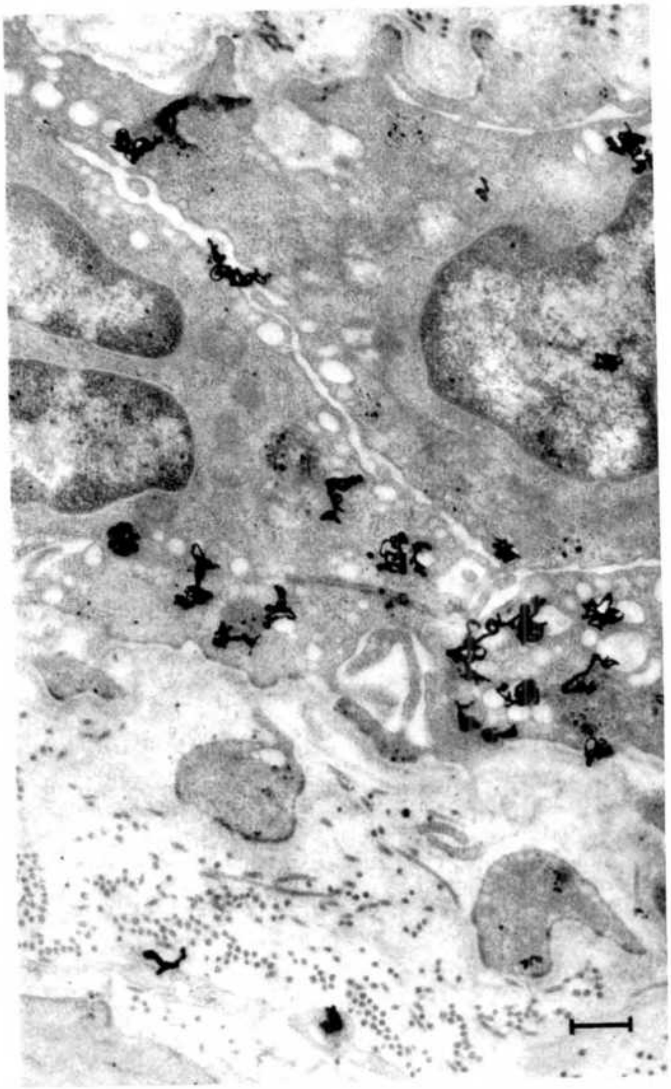

FIGURE 7. An autoradiograph of dermis showing radioactively labelled cells, but unlabelled collagen. Bar = 0·5 $\mu$m.

In keratinocytes from all samples, intracellular membranes, e.g. nuclear, mitochondrial and endoplasmic reticulum membranes, were not noticeably labelled. In melanocytes, however, the membranes surrounding individual melanin granules were heavily labelled in addition to plasma membrane labelling (Fig. 5). In Langerhans cells also there was a distinctive labelling pattern, with individual Langerhans granules and the plasma membrane bearing silver grains (Fig. 6).

The acellular part of the dermis consisting of collagen and elastin fibres was unlabelled. The cells in the dermis showed mostly plasma membrane label with a few grains in transit through the cytoplasm (Fig. 7).

## DISCUSSION

The small amount of fucose-containing epidermal glycolipid is soluble in water/ethanol mixtures and is thus removed during histological processing. Fucose is incorporated into glycoproteins by the Golgi apparatus of the cell (Bennett & Leblond, 1977) and is catalysed by the action of specific fucosyltransferases on guanosine-diphosphate-fucose (GDP-fucose). The autoradiographs showed that ($^3$H)-L-fucose was incorporated into components of the keratinocytes which, in normal epidermal tissue, were located predominantly in the cell plasma membrane (Table 1). Proportionately less ($^3$H)-L-fucose was associated with the plasma membranes of keratinocytes of uninvolved psoriatic epidermis and even less with the plasma membranes of keratinocytes of involved psoriatic epidermis.

It is a feature of involved psoriatic epidermis that the incorporation rate of metabolic precursors into epidermal components is usually higher than that into uninvolved psoriatic epidermis and much higher than that into normal epidermis and is indicative of a higher metabolic rate of involved psoriatic skin as a whole. However, it is clear from the present work that the metabolic rates of individual cells in involved psoriatic skin *in vitro* can show a very wide variation within the same tissue and unfortunately the practical limitations of the method prevent a sufficiently large number of cells from being assessed to define the limits of variability. Nevertheless, the difference in distribution pattern of ($^3$H)-L-fucose in keratinocytes of involved psoriatic epidermis was consistent irrespective of whether the total ($^3$H)-L-fucose incorporation into the involved psoriatic cell was less than or greater than the incorporation into parallel control samples of uninvolved psoriatic and normal keratinocytes and thus appeared to be independent of the metabolic rate of the tissue.

There are several possible explanations for the apparent decrease in the proportion of the total incorporated fucose associated with the plasma membrane. It may relate either to an incomplete synthesis of glycoprotein molecules, that is, molecules lacking terminal sugars, or to the loss of a complete glycoprotein. It is relevant that the incorporation of ($^3$H)-glucosamine, a precursor of terminal sialic acid, into membrane-bound glycoproteins of involved psoriatic epidermal cells was also less than its incorporation into those of uninvolved psoriatic and normal epidermal cells (King, Paul & Gray, 1979). These results suggest defects in the pathway to glycoprotein synthesis, for example, loss of glycosyltransferase activities. Equally possible would be an increase in the activities of catabolic enzymes like the glycosidases, which could remove sugars from the intact glycoprotein after its insertion in the plasma membrane. A further possibility is that the psoriatic cells may have a defect in the mechanism by which fully synthesized glycoproteins in the Golgi apparatus are transferred to and become part of the plasma membrane. It is also possible that the plasma membrane level of glycoprotein is normal, but that the cytoplasmic level is raised.

It is of interest to note that some of the properties of psoriatic cells seem similar to those of cultured cells transformed by virus. There is often a reduction in the terminal sugars of glycoproteins in the plasma membrane of transformed cells (Grimes, 1970; Den *et al*., 1971) which is associated with the loss of growth control. Some glycoproteins are deleted from the surface of transformed cells (Critchley, 1974) and the activities of glycosidases in some transformed cell lines are much higher than in the corresponding untransformed cells (Bosmann, Hagopian & Eylar, 1969). A correct explanation of our present results is likely to come from a more detailed study of the composition, structures and metabolism of the membrane-bound glycoproteins in normal and psoriatic epidermis. This is essential information in an area which in our opinion is possibly the most important in current research on psoriasis.

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# A controlled trial of photochemotherapy for persistent palmoplantar pustulosis

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## SUMMARY

Twenty-two patients with bilaterally symmetrical persistent palmoplantar pustulosis were treated on one randomly selected side with oral psoralen and long-wave ultraviolet light. The treated side cleared completely in twelve patients, almost cleared in five patients and improved in four. One patient improved on both sides.

Fifteen of the twenty-two patients were then treated with topical psoralen and long-wave ultraviolet light on the side that had previously been used as a control. Similar results were obtained. Seven patients cleared completely, six patients were much improved and two were improved.

These results are significantly different from those liable to occur by chance ($P<0{\cdot}001$).

Persistent palmoplantar pustulosis (P.P.P.) is a disease characterized by chronic recurring sterile pustulation of the palms and soles, with sequential change in the pustules from yellow to brown, followed by shedding of scale. The aetiology is not understood, and management is difficult. Recent reports (Mizuno, Vematzo & Ohno, 1976; Menné, 1976; Morison, Parrish & Fitzpatrick, 1978) on the benefit of photochemotherapy (PUVA) for P.P.P. have included small numbers of patients or have been uncontrolled. A pilot study of eighteen patients treated twice weekly with oral psoralen produced poor results, and a controlled trial was carried out to examine the role of PUVA in the management of P.P.P. Oral psoralen was compared with topical psoralen.

## PATIENTS AND METHODS

### *Patients*

Twenty-two patients with bilaterally symmetrical P.P.P. of at least 1 year's duration were referred from the out-patient department of St John's Hospital. The purpose of the trial was explained to them and their consent obtained. There were six males of mean age 47·8 years (s.e. 2·09) and sixteen females of mean age 52·9 (s.e. 2·47). The mean age of onset was 46·1 years (s.e. 2·1) and the duration of

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disease 5·3 years (s.e. 0·94). The feet were affected alone in thirteen cases, the hands alone in two cases and both together in seven cases.

### *Materials*

*Methoxsalen.* (1) 8-Methoxypsoralen (8-MOP) in 10 mg tablets (methoxsalen, Promedica, supplied by Stiefel Laboratories U.K. Ltd). Taken with food 2 h before irradiation according to body weight: 30–50 kg: 20 mg; 50–65 kg: 30 mg; 65–80 kg: 40 mg; 80–90 kg: 50 mg. (2) 0·15% 8-MOP in an oil emulsion supplied as Meladinine (Basotherm, West Germany).

*Radiation.* (1) Waldman PUVA 180 foot unit containing 12 515T8/PUVA Sylvania fluorescent tubes. (2) Waldman PUVA 200 hand unit containing 14 F8T5/BL Sylvania tubes.

These bulbs deliver high intensity ultraviolet light with a spectral emission of between 320 and 400 nm peaking at 365 nm. The output was measured with a Waldman PUVA meter and ranged from 5 to 7 $mW/cm^2$ at the estimated distance of the skin surface. The time needed to deliver the required dose was read off a table.

### *Methods*

All topical and systemic treatment was stopped 2 weeks before entry but during the trial emulsifying ointment BP was applied equally to both sides at least twice daily. For the initial and major part of the trial, oral psoralen was used, and patients were irradiated four times per week on one randomly selected side. The opposite side was covered and served as a control. Treatment days were Monday, Tuesday, Thursday and Friday. The initial dosage of ultraviolet irradiation was according to skin type as decided by personal history of burning and tanning with sun exposure and ranged from 0·5 $J/cm^2$ to 2 $J/cm^2$. Increments of 0·5–1 $J/cm^2$ were made at not less than 48 h intervals until clinical improvement was obtained, when it was held at that level, or until 1 h of irradiation had been given. Precautions for the protection of the eye were according to standard PUVA practice (Vella Briffa *et al.*, 1978). The end point was the completion of thirty treatments. Patients were seen weekly by a single observer (D.M.) and the extent of disease was charted on outline diagrams of the palms and soles. An assessment of both the treated and untreated side was also made on a horizontal visual analogue scale (Aitken, 1969):

| 0 | 25 | 50 | 75 | 100 |
|---|---|---|---|---|
| worse | no change | improved | much improved | clear |

Improved meant less pustulation, much improved meant no pustulation with residual erythema and scaling, but the system was flexible and allowed for intermediate positions of assessment on a continuous scale from 0 to 100.

Final assessment was made on completion of thirty treatments. Colour photographs were taken before and at the end of the trial for later assessment by an independent observer.

The significance of the differences in response between the two sides was tested using the Wilcoxon matched pairs signed-rank test (Siegel, 1956).

In the subsequent part of the trial, the side that had been used as a control was treated with topical psoralen for a further thirty treatments. 8-MOP emulsion was painted on to affected areas only, using cotton wool on a stick, 1½ h prior to irradiation. The side that had already been treated with UV-A and oral psoralen was maintained with the same emulsion.

TABLE 1. Results of treatment with oral psoralen and PUVA

| | Cleared | Much improved | Improved | No change | Worse | Total |
|---|---|---|---|---|---|---|
| Treated side | 12 | 5 | 5 | 0 | 0 | 22 |
| Untreated side | 0 | 0 | 13 | 6 | 3 | 22 |

## RESULTS

The results of treatment with UV-A and oral psoralen are shown in Table 1, and illustrated in Fig. 1.

Twelve patients cleared. Five patients were much improved apart from residual scaling, or the occasional isolated pustule. Four patients improved, and one patient showed some improvement on both sides, but no difference between them. Apart from the latter, the treated side did better in every case. The mean treatment dose at clearing for the soles was $12 \pm 1{\cdot}20$ (s.e.mean) J/cm$^2$ and for the palms was $10{\cdot}5 \pm 1{\cdot}44$ (s.e.mean) J/cm$^2$. In most cases, improvement was not seen until between ten

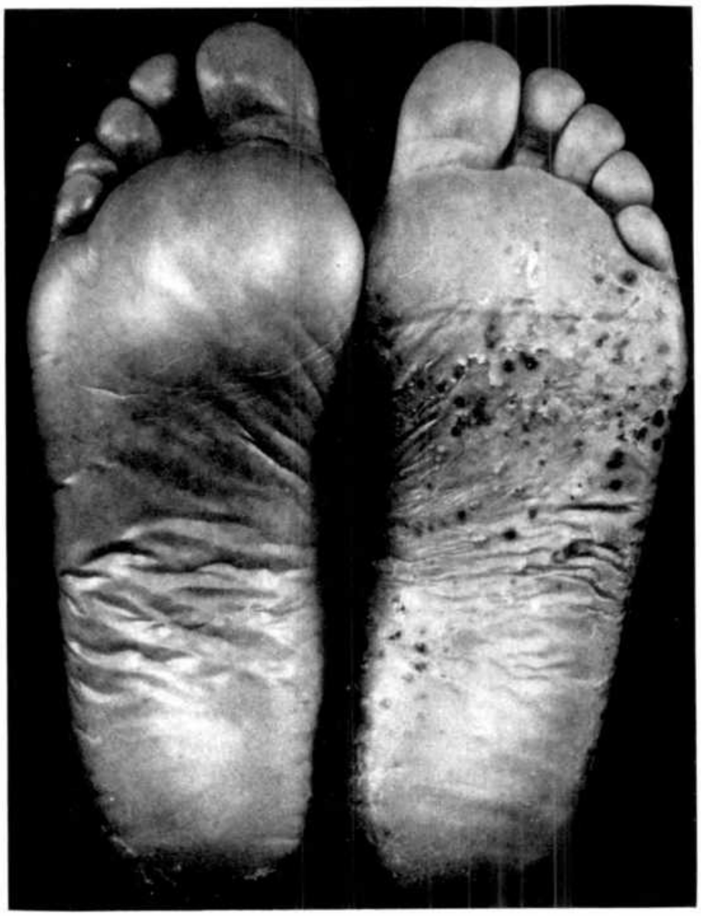

FIGURE 1. Results of treatment with UV-A and oral psoralen on treated and untreated soles.

TABLE 2. Result of treatment with topical psoralen and UV-A

| Cleared | Much improved | Improved | No change | Worse | Total |
|---|---|---|---|---|---|
| 7 | 6 | 2 | 0 | 0 | 15 |

and twenty treatments had been given, and the mean number of treatments to clear was twenty-six (range 18–30).

The difference in response between treated side and untreated is significant ($P < 0{\cdot}001$).

Photographic records were available for twenty patients and were independently assessed. Agreement was good between the two observers, and this method gave the same comparative result as the visual assessment.

The results of treatment with topical psoralen are shown in Table 2.

Fifteen patients were given thirty treatments with similar results to those obtained with oral psoralen. Seven cleared, all of whom had previously cleared with oral psoralen. Six were much improved, including five who had cleared on the opposite side with oral psoralen. Two were improved, achieving a result similar to those on oral psoralen.

The mean dose at clearing for topical psoralen was $8{\cdot}05$ J/cm$^2$. No statistical difference could be shown between the response to oral psoralen when compared with topical, but the numbers in the latter group are too small for reliable comparison.

### *Maintenance*

Of the original twenty-two patients, six remain clear and off all treatment for 1 month (1), 2 months (3) 6 months (1) and 10 months (1). This was after a gradual reduction in frequency of maintenance until it could be stopped. The frequency was reduced to twice weekly for 4 weeks and then to once weekly for a further 4 weeks before stopping.

Three patients remain clear and attend weekly but cannot tolerate a reduction in the frequency of maintenance without relapse. All are on oral psoralen.

Nine patients are greatly improved and on weekly maintenance. Two attend every 2 weeks and two were lost to follow-up. Of these patients eight are on oral psoralen and one is on topical. Most patients converted back to oral photochemotherapy for reasons to be discussed.

### *Side-effects*

Four patients were burned with the topical psoralen, often because of accidental painting of previously untreated skin. One patient was burned with oral psoralen.

Four patients experienced nausea after taking oral psoralen and four patients had mild ankle swelling, again with oral psoralen. Six patients developed a brief non-purulent conjunctivitis in one eye at variable times during treatment with oral psoralen. No obvious explanation was found.

## DISCUSSION

This study has shown that photochemotherapy is effective for palmoplantar pustulosis. Treatment was given frequently because of the poor results of twice weekly irradiation in the pilot study. Improvement may not be seen until at least twenty treatments have been given and both the number of treatments to clear, and the dose of irradiation at clearing, were higher than for plaque psoriasis treated with whole body PUVA. As a consequence, the cumulative dosage of irradiation at clearing was approximately twice that for whole body PUVA. Frequent maintenance was also needed with early relapse if it was stopped. Reduction in the frequency of maintenance may result in a partial relapse, but further deterioration is prevented if treatment is continued. Symptomatic relief is an early feature and often stressed by those patients with residual but milder disease. A few patients have been able to stop treatment after a gradual reduction in maintenance. These may be spontaneous remissions which are, however, uncommon in P.P.P. (Ward, Corbett & Hanna, 1976) and persisting unremitting disease had been present for 1–10 years (mean 5·3 years). Follow-up will determine how many subsequently relapse. No statistical difference has been shown between the response to oral and topical psoralen, but five patients who had cleared with oral psoralen failed to clear with topical. However, the mean dose of UV-A was lower in the topical group. Some patients, especially the less agile, were unable to use the topical preparation accurately, and burns were seen due to inadvertent painting of previously untreated skin. The same difficulty arose when the disease margin extended, or new areas appeared. Painting of the whole of the palm or sole is perhaps a less hazardous method of using topical psoralen. Pigmentation tended to be irregular with topical psoralen, making dosimetry difficult.

Oral psoralen was more convenient, pigmentation was regular and burns were infrequent. Dosimetry was more easily controlled.

Light sources are needed that will deliver a uniform irradiance, not only to the soles, but also to the backs of the heels and the sides of the feet where patches tended to persist. Not only were these areas difficult to irradiate but clearance of plantar disease seemed to encourage marginal pustulation that had not been present before. Patients with classical P.P.P., with well separated brown pustules, responded better than those with dense small pustules intermingled with vesicles. Those with thick scaling were also more resistant. A combination of PUVA with other forms of treatment may allow a reduction in the frequency of maintenance. These possibilities are being studied.

## ACKNOWLEDGMENTS

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# A comment on the routine testing of sunglasses in photochemotherapy

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## SUMMARY

An estimate of the UV transmission of sunglasses is often made in Dermatology Departments by employing the irradiation source used for PUVA therapy and a UV-A meter. The object of making these measurements is to assess the UV opacity of the glasses in natural sunlight. The present communication examines the adequacy of this simple experimental technique and concludes that, in general, the results obtained are unlikely to lead to a misleading impression.

One of the undesirable side-effects of photochemotherapy (PUVA) when the psoralens are administered orally is the theoretical risk of the induction of cataracts (Parrish *et al.*, 1978). Although protective goggles are worn during treatment, it is a wise precaution to shield the eyes for several hours after psoralen ingestion since the UV-A levels in natural sunlight on clear days in the U.K. can be similar to the levels encountered in treatment units. Complete eye protection may be achieved by wearing glasses which are both opaque to ultraviolet radiation and incorporate side protection, e.g. Blak-Ray Contrast Control Spectacles Model No. UVC-303 (Ultraviolet Products Inc.). However, many patients object to wearing these glasses for cosmetic reasons and prefer to wear commercial sunglasses. Also economic considerations may prevent all patients being given a pair of Blak-Ray spectacles.

An estimate of the UV transmission of sunglasses is often made in Dermatology Departments by employing the irradiation source used for PUVA therapy and a UV-A meter. Indeed this technique has been described by Wennersten (1978). Since the object of making these measurements is to assess the UV opacity of the glasses in natural sunlight, the purpose of the present communication is to examine whether this simple experimental technique is adequate.

## MATERIALS AND METHODS

The fraction of natural UV radiation transmitted through the lens of a pair of spectacles is given mathematically by

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$$\int_{UV} G(\lambda)T(\lambda)d\lambda \Big/ \int_{UV} G(\lambda)d\lambda \tag{1}$$

where $G(\lambda)$ is the spectral irradiance in mW cm$^{-2}$nm$^{-1}$ of global radiation under approproate conditions (e.g. time of day, day of year, geographical latitude, and so on) at a wavelength of $\lambda$nm, and $T(\lambda)$ is the fractional transmission of the lens at wavelength $\lambda$. The integration is over all wavelengths in the UV up to 400 nm. The apparent fractional transmission of UV through the spectacle lens when a PUVA lamp and UV-A meter are used can be expressed by

$$\int_{UV} P(\lambda)D(\lambda)T(\lambda)d\lambda \Big/ \int_{UV} P(\lambda)D(\lambda)d\lambda \tag{2}$$

where $P(\lambda)$ is the spectral irradiance in mW cm$^{-2}$nm$^{-1}$ of the PUVA lamp at wavelength $\lambda$, and $D(\lambda)$ is the relative spectral response of the UV-A meter.

Strictly speaking both the numerator and denominator of Equations 1 and 2 should incorporate a term expressing the action spectrum for ocular damage due to psoralen photosensitization. However this action spectum is uncertain and even if it were known it is unlikely that it would alter the conclusions of this paper.

For the purpose of this communication, values for the spectral distribution of natural UV radiation, $G(\lambda)$, are taken to be the mean values for the English summer derived from Bener (1972). In a recent survey of the UVR emissions from photochemotherapy units (Diffey, Challoner & Key, 1980) it was found that the relative spectral emission of the treatment lamps was the same for all units studied. This spectral irradiance, $P(\lambda)$, is shown in Fig. 1 for a UV-A irradiance of 4·0 mW cm$^{-2}$. The global spectrum, $G(\lambda)$, is also shown in Fig. 1 for comparison. The integrated global UV irradiance is 4·4 mW cm$^{-2}$. The spectral response, $D(\lambda)$, of a typical UV-A meter (International Light IL442A phototherapy radiometer) is shown in Fig. 2.

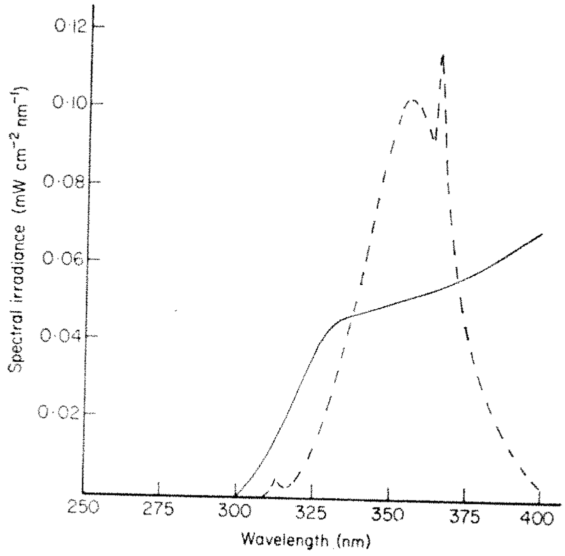

FIGURE 1. Spectral irradiance in the ultraviolet. PUVA lamp (- - - - - -); global UV radiation in English summer (———).

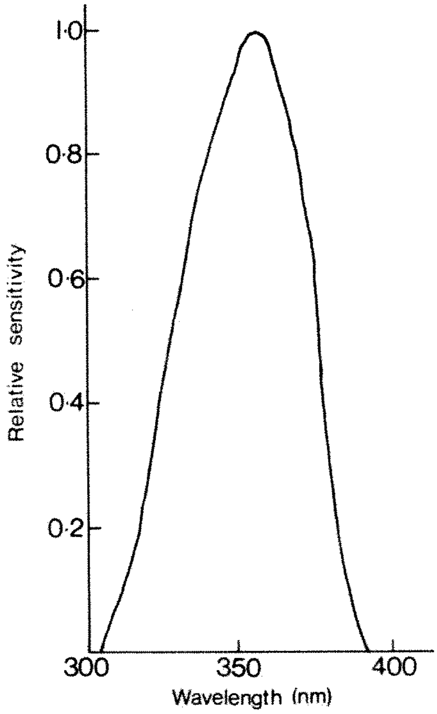

FIGURE 2. Spectral response of a typical UV-A meter.

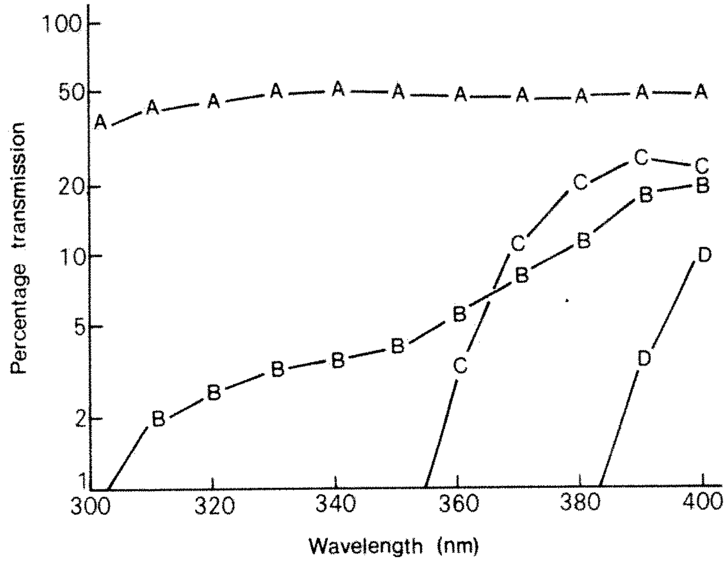

FIGURE 3. Spectral transmission curves of four pairs of sunglasses (see Table 1 for details).

TABLE 1. Global UV transmission compared with apparent transmission measured with PUVA lamp and UV-A meter

| | | Percentage transmission | |
|---|---|---|---|
| Curve | Type of Sunglass | Global UV (Equation 1) | PUVA lamp (Equation 2) |
| A | Fashion—CR39* lens | 50 | 51 |
| B | Clip-on—tinted lens | 8·7 | 5·3 |
| C | Children's—CR39 gradient lens | 10·3 | 3·8 |
| D | Fashion—polarized lens | 1·3 | 0·3 |

* CR39 is a shatterproof, plastic material.

## RESULTS

The spectral transmission of four widely-available commercial sunglasses was measured using a xenon arc lamp, a single grating monochromator and a collimated photodiode. The observed transmission curves, $T(\lambda)$, are illustrated in Fig. 3. The results of numerically integrating Equations 1 and 2 for each of these pairs of sunglasses are presented in Table 1. In general the PUVA lamp/UV-A meter method underestimates the global UV transmission, particularly when the transmission spectrum falls rapidly as the wavelength decreases below 400 nm. This is illustrated by sunglasses C and D in Table 1 where the PUVA lamp/UV-A meter combinations indicate only 37% and 23% of the true global UV transmission respectively. However, the absolute values of transmission that would be indicated experimentally probably do not lead to inappropriate conclusions.

The reason for this underestimate is due to the low amplitude of the PUVA spectrum and, more importantly, the insensitivity of the UV-A meter in the range 380–400 nm. To highlight this effect, consider a pair of sunglasses which have 100% transmission from 400 to 380 nm and zero transmission for all wavelengths less than 380 nm. In this case the global UV transmission as calculated by Equation 1 would be 28% whereas the PUVA lamp/UV-A meter would indicate only 0·7% transmission; a factor of 40 in relative transmission. Although this example is probably unrealistic, it serves to illustrate the error that could theoretically occur. Nevertheless it is concluded that the simple technique of evaluating the UV opacity of sunglasses with a PUVA lamp/UV-A meter is reasonable and is unlikely to yield a misleading impression.

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# Endogenous peroxidases: a morphological and functional marker to study inflammatory skin diseases

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## SUMMARY

The visualization of peroxidase positive lysosomes by diaminobenzidine plus $H_2O_2$ allowed easy identification of neutrophils, eosinophils, monocytes and young tissue macrophages in 0·2 μm to 0·5 μm skin plastic sections of large surface area. Evaluation of the lysosomal functions of these phagocytic cells was greatly improved by the possibility of correlating observations on the same cell at light and electron microscopic levels.

The study of inflammatory processes induced in normal skin or due to skin diseases requires identification as well as kinetic and functional studies of the cells involved.

The visualization by diaminobenzidine tetrachloride (DAB) plus $H_2O_2$ of the peroxidase-positive lysosomes of polymorphonuclear leukocytes, of eosinophils, of monocytes and of young macrophages permits an exact identification of these cells and an evaluation of the functional activity of their lysosomal systems (Breton-Gorius & Reyes, 1976).

These studies may be performed at the light microscopic level on smears or tissue prints, or, more usually, at the ultrastructural level (Breton-Gorius & Reyes, 1976; Blinzinger *et al.*, 1978). On 3–5 μm thick histological paraffin sections the staining of cells is too dark and, as a consequence, identification of cells is impossible.

We describe here a new technique using DAB staining as a morphological and functional marker to study inflammatory cells in histological sections by light and electron microscopy. This technique, performed on 0·2–0·5 μm sections of large surface area, permits the identification of neutrophils, eosinophils, basophils, monocytes and young tissue macrophages. This identification is possible even if the nucleus of the cell is not visible in the section and also if the cell has been destroyed and has released its lysosomes into the extracellular spaces.

## MATERIALS AND METHODS

### *Biopsies*

Punch biopsies (6 mm) were obtained after local anaesthesia with 2% xylocaine without adrenalin.

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### *Fixation*

The biopsy was placed in 10 ml of Karnovsky's fixative (prepared less than 8 days before), and diluted by half in 0·1 mol/l cacodylate buffer at pH 7·4 just before use (Karnovsky, 1967). After 15 min of fixation, the biopsy was cut into slices of 0·4 mm thickness, perpendicular to the cutaneous surface, with a newly designed hand microtome (Dubertret *et al.*, 1979). The total duration of the fixation was 5 h at 4°C. The fragments were then washed twice for 15 min and subsequently for 12 h at 4°C in cacodylate buffer (0·1 mol/l, pH 7·4).

### *Cytochemical techniques*

After fixation the fragments were incubated for 12 h in the dark at 4°C in Graham & Karnovsky (1966) medium without $H_2O_2$. Then they were incubated for 1 h in the dark at room temperature in freshly prepared complete Graham & Karnovsky (1966) medium. It consisted of a solution of 5 ml of 3,3′-diaminobenzidine tetrachloride (Sigma) in 10 ml of 0·05 mol/l Tris-HCl buffer (pH 7·6) to which 0·1 ml of freshly prepared 1% solution of $H_2O_2$ had been added just before use. The fragments were then washed twice for 15 min in cacodylate buffer (0·1 mol/l, pH 7·4).

Controls of the specificity of the reaction were performed by incubating one fragment in 2% catalase (C-100 Sigma) in 0·05 mol/l Tris-HCl buffer (pH 7·6) for 6 h at room temperature. Thereafter this fragment was put in Graham & Karnovsky's (1966) medium without $H_2O_2$ with 2% catalase (C-100 Sigma) for 12 h at 4°C and subsequently 1 h at room temperature. Another fragment was treated with the same protocol followed by three washes of 30 min each in 0·05 mol/l Tris-HCl buffer (pH 7·6). Then this fragment was put in $10^{-3}$ mol/l $FeCN_6K_4$ (Merk) in 0·05 mol/l Tris-HCl buffer (pH 7·6) for half an hour followed by two washes in 0·05 mol/l Tris-HCl buffer and one wash in cacodylate buffer (0·1 mol/l, pH 7·4). In these two controls the inhibition has to be complete.

An optional post-fixation with $OsO_4$ for 2 h at room temperature permitted, if desired, a more intense staining with DAB for ultrastructural investigations. This was done as previously described (Dubertret *et al.*, 1979).

### *Inclusion*

The resin used was that previously described (Dubertret *et al.*, 1979). It was slightly hardened because polymerized DAB may be difficult to cut. The epon mixture contained: 17 g V.C.D. (vinyl cyclohexene dioxide Ladd); 43 g Epon 812 (Ladd); 67 g N.S.A. (nonenyl succinic anhydride Ladd).

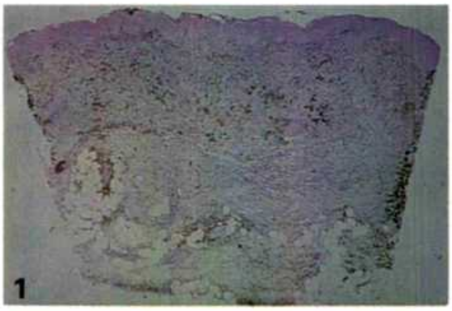
Inclusion, orientation and polymerization, sectioning for light and electron microscopy, staining for light microscopy and photography were performed as previously described (Dubertret *et al.*, 1979).

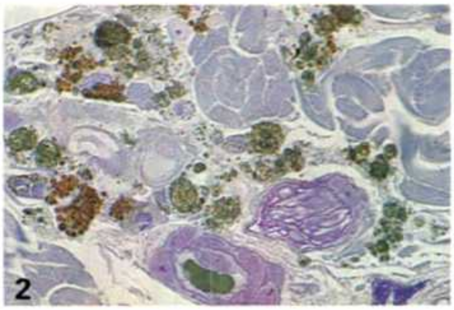
## RESULTS

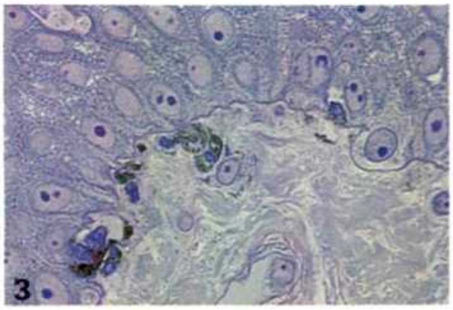
Using a (×2·5 fold) scanning objective for a 0·5 μm large surface area section the DAB staining was clearly visible (Fig. 1).

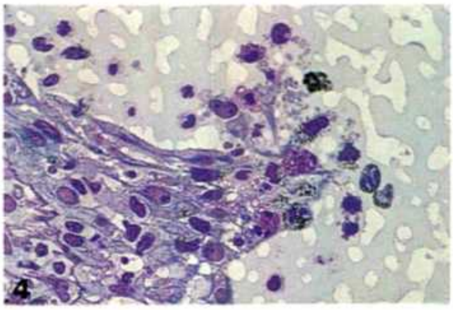
At a stronger magnification a precise identification of inflammatory cells was possible. Neutrophils were full of numerous little dark granules, black or green (Figs 2–6). When neutrophils had burst as in leukocytoclasis (Fig. 2) it was possible to recognize numerous fragments of cells scattered around the vessels.

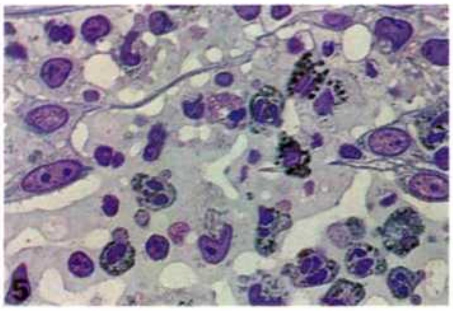
Eosinophils were easily recognized because they possessed numerous positive granules of equal size but clearly bigger than those of neutrophils. The peroxidase of eosinophil granules is different from the peroxidase of neutrophils (see for review: Breton-Gorius & Reyes, 1976). The peroxidase of eosinophils gave an orange staining with DAB clearly different from the black or green staining produced with DAB in neutrophils and monocytes. This peculiar staining, very convenient for identify-











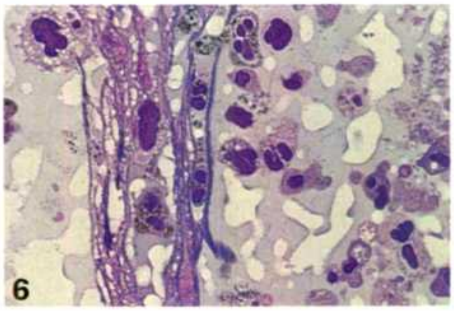


FIGURE 1. A 0·5 μm section of acute haemorrhagic oedema stained by Giemsa in acetified water. Dark deposits are DAB staining of peroxidase positive cells. They are clearly visible (× 10).

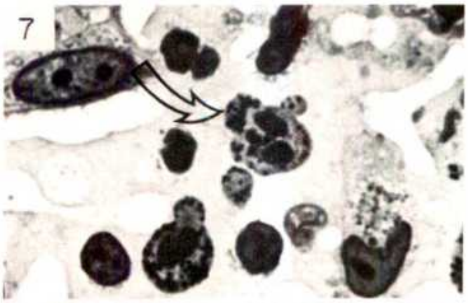
FIGURE 2. Part of Fig. 1, $OsO_4$ was omitted. The granules of eosinophils are brown and their refracting crystal can be recognized. Peroxidase positive granules of neutrophils are black-green. Neutrophils are burst or dying. On the right it is possible to see some extracellular granules of eosinophils. Below, a blood vessel is seen with some red cells stained in green by DAB (× 400).

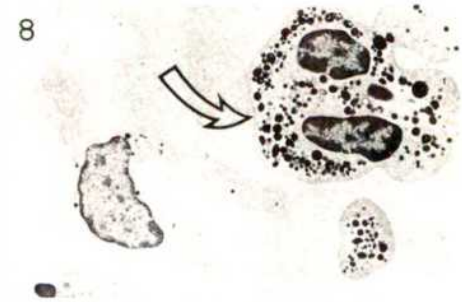
FIGURE 3. A 0·5 μm section of bullous pemphigoid stained by May-Grunwald Giemsa without $OsO_4$. Along the basement membrane, on the left one can detect an eosinophil and a neutrophil; in the middle of the slide two neutrophils are in contact with a degranulating mast cell. On the right hand side, a melanocyte is seen which is clearly peroxidase negative (× 400).

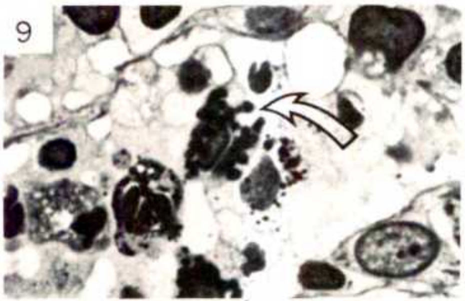
FIGURE 4. Epidermis between two confluent vesicles in contact dermatitis. Post-fixation with $OsO_4$; staining with Giemsa in acetified water. The basement membrane is seen to the left. In the top of the epidermis are two purple basophils, one neutrophil adjacent to a group of peroxidase positive granules (neutrophils ?). In the vesicle there are four purple degranulating basophils, two neutrophils with little black granules and one eosinophil with big black granules and whose nucleus cannot be seen (× 250).

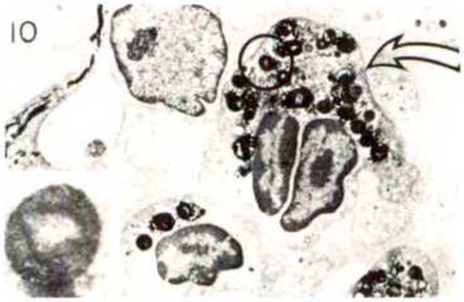
FIGURE 5. On the right a typical eosinophil and just below another eosinophil. In the middle there is a purple basophil. Down, in the middle, a tissue macrophage peroxidase positive. Numerous neutrophils are present in all of this section (× 400).

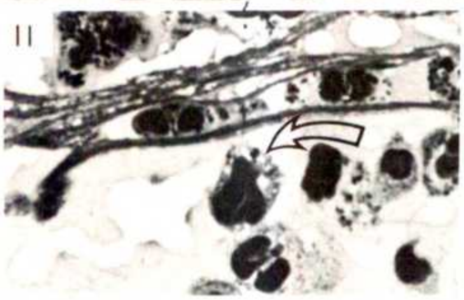
FIGURE 6. The same section as Fig. 4. In the centre, close to two neutrophils, is a tissue macrophage with only a few peroxidase positive granules (× 400).











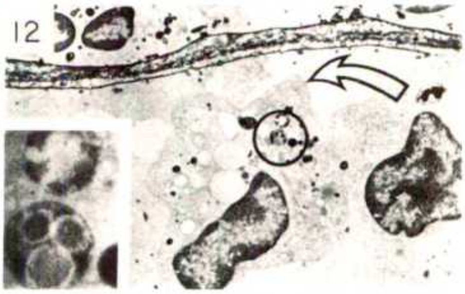


FIGURE 7. Part of Fig. 5. The arrow shows a typical neutrophil. Down on the right a tissue macrophage can be seen (× 1400).

FIGURE 8. The same neutrophil as in Figs 5 and 7 under electron microscopy. Close to it, is a tissue macrophage (× 3300).

FIGURE 9. Part of Fig. 5. The arrow shows a typical eosinophil (× 1400)

FIGURE 10. The same eosinophil as in Figs 5 and 9 under electron microscopy. The inset show the eosinophil granule situated in the circle (× 3300).

FIGURE 11. Part of Fig. 6. The arrow shows a typical tissue macrophage (× 1400).

FIGURE 12. The same tissue macrophage as in Figs 6 and 11, but under electron microscopy. The inset shows the phagolysosome situated in the circle (× 3300).

ing eosinophil granules (Figs 2 and 3), disappeared when $OsO_4$ post-fixation was used (Figs 4 and 5). In this last situation all the peroxidase positive granules were black as a consequence of the osmiophilia of oxidized DAB. When eosinophils burst, their granules are scattered in the intercellular space (Fig. 2).

Basophils were easily recognized by their typical purple colouration (Figs 4 and 5). The peroxidase activity of basophil granules was usually not observed in tissues after the fixation described.

Monocytes and young macrophages were easily distinguished from neutrophils because they possess a one-lobed nucleus and less peroxidase positive granules of irregular size (Figs 5–7, 11, 12) as described by Daems *et al.* (1975), Breton-Gorius & Reyes (1976) and van der Rhee *et al.* (1979a). As circulating monocytes differentiate into young macrophages and mature macrophages, the number of peroxidase positive granules decreases and finally they disappear (Figs 5–7, 11, 12) as described by Daems *et al.* (1975) and van der Rhee *et al.* (1979a). Usually mature macrophages and epithelioid cells contain a number of cytoplasmic peroxidase negative granules, visible on 0·5 $\mu$m sections of large surface area (Dubertret *et al.*, 1979), allowing them to be distinguished from lymphoid cells (van der Rhee *et al.*, 1979a). As noted by van der Rhee *et al.* (1979b) we did not observe peroxidase positivity in rough endoplasmic reticulum or in the nuclear envelope of skin macrophages.

Red cells were orange-green after DAB staining as a result of the pseudo peroxidase activity of haemoglobin (Fig. 2) (Lewis & Knight, 1977).

Cells of normal skin did not manifest peroxidase activity. In the conditions described here melanocytes were peroxidase negative (Fig. 3).

In order to prove the exactitude of the light microscopic observations, light and electron microscopy was performed on the same neutrophils (Figs 5, 7, 8), the same eosinophil (Figs 5, 9, 10) and the same macrophage (Figs 6, 11, 12). It can be seen that the different cell types identified in the electron microscope correspond to those identified under light microscopy.

## DISCUSSION

To obtain a complete reaction with DAB it was necessary to incubate tissue slices (0·4 mm thick sections of a 25 mm$^2$ surface area) overnight at 4°C in Graham-Karnovsky medium without $H_2O_2$ because the penetration of DAB into tissue samples was not very quick. The omission of $H_2O_2$ during the first incubation step allowed better tissue preservation and avoidance of diffusion artefacts. The peroxidation of DAB caused very dense deposits which prevented the distinguishing of peroxidase positive cells from each other on tissue sections thicker than 1 $\mu$m. Thus, better results were obtained by making 0·2–0·5 $\mu$m sections. To obtain these very fine sections of large surface area it was necessary to follow the exact protocol as described in material and methods.

Peroxidation of DAB can be induced not only by different enzymes such as peroxidases and catalases (Roels *et al.*, 1975) but also by cytochrome, haemoglobin (Lewis & Knight, 1977), lipofuschin (Breton-Gorius, 1974), noradrenalin and melanin (Novikoff *et al.*, 1971). Using the fixation, the pH and the concentration of $H_2O_2$ as used by Breton-Gorius (1974) only endogenous peroxidases and haemoglobin produced the peroxidation of DAB. Under these technical conditions, catalases, noradrenalin and melanin are known not to give positive reactions with DAB (Breton-Gorius, 1974; Novikoff *et al.*, 1971). The possible positivity of lipofuschin is to be investigated.

The pseudo peroxidase activity of haemoglobin was very convenient because it allowed visualization of extravascular red cells.

Controls of specificity were slightly different from those described by Breton-Gorius (1974). In fact, the omission of $H_2O_2$ was not sufficient to inhibit peroxidase activity as a consequence of endogenous production of $H_2O_2$, particularly in eosinophils and in monocytes even after aldehyde fixation of skin. Preincubation of fragments with catalase was necessary to obtain a complete enzymatic inhibition. This may be due to the quick diffusion of DAB in skin samples and to the slower diffusion of bovine catalase.

DAB staining seems to be a very convenient morphological marker to identify, in skin, inflammatory cells originating from bone marrow via the blood stream (Breton-Gorius & Reyes, 1976). In these cells peroxidases are present in some lysosomal granules. Thus DAB staining is also a marker for lysosomal functions in these inflammatory cells (Breton-Gorius & Reyes, 1976). Lysosomes play a very important role in the functions of neutrophils (Goldstein, 1976), eosinophils, monocytes and macrophages (Cochrane, 1975). They contribute to phagocytosis and bacterial killing. Lysosomal hydrolytic enzymes may be released from cells and induce tissue injury. Lysosomes seem also to be able to kill tumour cells (Bucana *et al.*, 1976). All these functions of peroxidase positive lysosomes can be studied in close correlation under light and electron microscopy with the method described.

In conclusion, DAB staining seems to be, under the technical conditions and with the controls described here, a very convenient method to study the morphological, kinetic and functional aspects of cells in inflammatory skin diseases. The possibility of correlating light and electron microscopy increases greatly the possibility of analysis. This technique may be useful not only for skin but also to study inflammatory processes in other tissues.

## ACKNOWLEDGMENTS

We are greatly indebted to Dr D.Averbeck for careful correction of this manuscript.

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# Erratum

The plate reproduced below is the correct version of Figs 2–7 in 'A simple method for correlating observations on skin at the light and electron microscope levels' by L.Dubertret, B.Bertaux, M.Fosse, F.Boulvin and R.Touraine published in Volume 102, pp. 149–154.

The original plate was printed upside down.

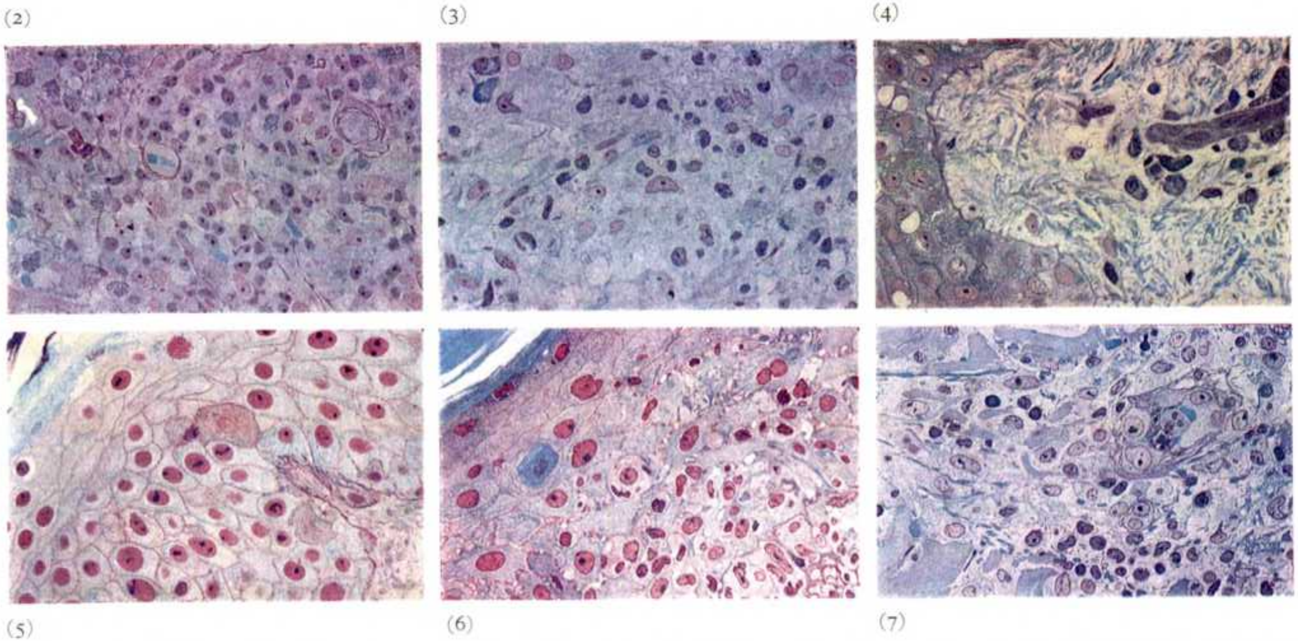

FIGURE 2. Alcoholic eosin-slow Giemsa (× 375). Dermal perivascular infiltrate. Note the basal membrane of capillaries, the purple basophil on the left and the numerous eosinophils above and below it. FIGURE 3. Alcoholic eosin-slow Giemsa (× 375). Chronic and polymorphic dermal infiltrate (mycosis fungoides). Note the numerous Lutzner cells. At the top numerous eosinophil leukocytes and two plasma cells on the left. The collagen is slightly basophilic. FIGURE 4. May-Grunwald Giemsa (× 375). The staining is less subtle than in Figs 2 and 3 but with more contrast. Two plasma cells are clearly seen under the capillary. FIGURE 5. Giemsa in acetified water (× 375). Two mitoses and a cell with a cerebriform nucleus in the epidermis. Note the strong staining of the basement membrane of the epidermis and of the intercellular substance joining keratinocytes. FIGURE 6. Giemsa in acetified water after post fixation with $OsO_4$ (× 375). A strong epidermotropism. Note the precision of cellular details, the strong staining of keratin, keratohyalin and of the intercellular substance joining keratinocytes. Down on the right the basement membrane of the epidermis. FIGURE 7. Giemsa in acetified water after post fixation with $OsO_4$ and 1% periodic acid for 30 min (× 375). A perivascular infiltrate. Note the purple basophil leukocyte and the trinucleated polymorphonuclear in the vessel, the binuclear eosinophil leukocyte on the left.

*British Journal of Dermatology* (1980) **102,** 675.

# Calcification of cultured urticaria pigmentosa skin

## AN ELECTRON OPTICAL STUDY OF THE CALCIUM-APATITE DEPOSITION

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### SUMMARY

Mast cells participate in experimental calcinosis. Skin from patients with urticaria pigmentosa (cutaneous mastocytosis) calcifies *in vitro*. In the present report, the nature of the calcium deposit was studied by electron optical techniques. The cultured mast cells of urticaria pigmentosa skin were surrounded by a large number of round bodies with an average diameter of 200–300 nm. Most of the round bodies were membrane bounded, while a few contained lamellar structures. These bodies may represent matrix vesicles as seen in calcifying hard tissues and/or may be remnants of mast cell granules. The calcified deposits were located on the round bodies, collagen fibrils and elastic matrix. Electron diffraction and X-ray microanalysis demonstrated that the deposits consisted of crystalline calcium apatite.

Systemic mastocytosis is frequently accompanied by osteosclerosis of the skeleton, while no soft-tissue calcification has been reported (Sagher & Even-Paz, 1967). Histological examination of the affected bone showed accumulation of mast cells in the osteosclerotic areas (Sagher & Even-Paz, 1967). Selye & Gabbiani (1964) reported that experimentally induced skin calcification in rats could be initiated by discharged mast cell granules. These findings point to a possible role of mast cells in calcification.

In a previous report, we demonstrated that skin from patients with urticaria pigmentosa (cutaneous mastocytosis) mineralized *in vitro* (Hentzer & Kobayasi, 1967). In the present work, involved skin from patients with urticaria pigmentosa (UP) and skin from healthy individuals were cultured under identical conditions and studied by bright- and dark-field transmission electron microscopy, selected area electron diffraction and X-ray microanalysis. The purpose of this study was to describe the ultrastructure and identify the nature of the mineralized deposits.

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## MATERIAL AND METHODS

Skin biopsies were obtained from lesions of six patients all suffering from urticaria pigmentosa (cutaneous type). Skin biopsies from healthy individuals of comparable sex and age were obtained from the Department of Plastic Surgery, Rigshospital, Copenhagen.

The culture medium used was Eagle's minimum essential medium with Earle's salts (GIBCO), enriched with 20% heat-inactivated fetal calf serum (GIBCO) and supplemented with 100 μg/ml penicillin, 50 μg/ml streptomycin and 50 μg/ml gentamicin (GIBCO). In the final medium, the total concentration of calcium was 1·98 mmol/l and phosphorus 1·14 mmol/l. The pH of the medium was buffered at 7·4 with 1 M Hepes (GIBCO).

Organ culture dishes (Falcon) with steel grids were used. Each biopsy was cut into small explants of approximately 2–4 $mm^3$. Four explants were placed on the steel grid of each dish. Approximately 2 ml of medium was added to each dish just covering the surface of the explants. The medium was changed twice a week during the culture period. The organ culture dishes were placed in an Ehret incubator gassed with humidified atmospheric air supplemented with 5% $CO_2$. The temperature was maintained at 37±0·5°C. The explants were harvested after 1, 2 and 3 weeks of culture. Before cultivation, explants were taken as controls of each patient. Altogether, ninety explants were studied.

Bright-field electron microscopy of the explants before and after culture was performed on all individuals. The explants were fixed in 3% glutaraldehyde in cacodylate buffer pH 7·4 with 7·5% sucrose for 1 day and post-fixed in 1% osmic acid for 1 h. The specimens were dehydrated in a series of ethanols of increasing concentrations and finally embedded in Epon 812. Ultrathin sections stained with lead citrate and uranyl acetate were examined with transmission electron microscopes of Siemens (Elmiskop IA) and Jeol (100 U).

### *Electron diffraction and dark-field electron microscopy*

Selected area diffraction patterns were recorded from stained as well as unstained ultrathin sections. The diffraction constant of the electron microscope was obtained as a function of the ring diameter from the diffraction pattern of vacuum deposited polycrystalline gold films (Johnson & Otkjær Nielsen, 1978). Orientational correlation between micrographs and diffraction patterns was made under appropriate consideration of image rotations. The image rotation relative to the diffraction pattern was determined as a function of magnification from double exposures of micrographs and selected area diffraction patterns of platelets of $MoO_3$ (Hirsch *et al.*, 1965).

Tilted-beam dark-field micrographs from the crystalline deposits were recorded using appropriate segments of the diffraction rings for imaging (Heidenreich, 1964).

Selected area diffraction and tilted-beam dark-field microscopy were made with a Jeol 100 U transmission electron microscope operated at 100 kV acceleration.

### *X-ray microanalysis*

The elemental composition of the calcified deposit was determined by electron-induced X-ray fluorescence microanalysis in a Jeol 100 C 'TEMSCAN' electron microscope equipped with a Si(Li) energy despersive detector. A beryllium window covering the detector renders possible only the detection of elements heavier than sodium.

X-ray spectra were recorded from unstained ultrathin sections mounted on 200 mesh copper grids coated with carbon. An acceleration voltage of 100 kV was used. Counting times of 100 s were selected.

Control spectra were obtained from areas of the sections without deposit.

## RESULTS

### *Bright-field electron microscopy*

The dermal cell infiltrate of UP skin was composed mainly of mast cells which were present during the entire cultivation period (Fig. 1). After the first week of culture, a large number of round bodies appeared in the surroundings of mast cells. Most of these bodies were membrane bounded and had an average diameter of 200–300 nm. Some of the bodies contained a granular material of varying electron density, while others appeared empty. Lamellar structures, as seen in mast cell granules, were demonstrated in a few round bodies.

During the second week of culture, an electron-dense crystalline deposit was found in the connective tissue of the UP skin explants (Figs 1 and 3). The deposits were located on the round bodies (Fig. 2), collagen fibrils and elastic fibres (Figs 3, 4b, 5b, 6b). Inside the round bodies, the deposit appeared as small needle-like crystallites with a random distribution (Fig. 2). The crystallites located on the collagen fibrils were arranged in a texture with most crystallites having their needle axis parallel to the axis of the individual collagen fibrils (Fig. 5b). Accentuation of the collagen fibril banding by the crystallites was present in areas with marked mineralization. The deposits within the elastic fibres

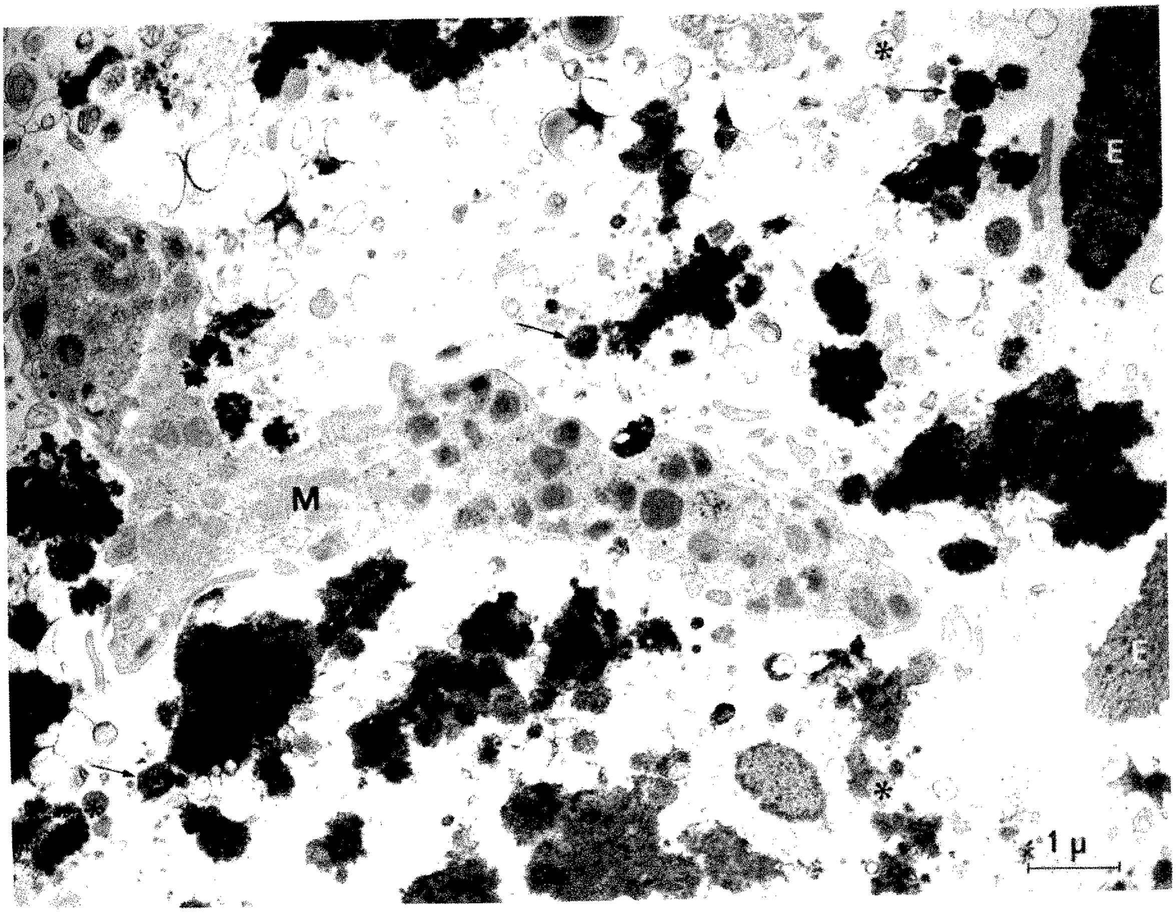

FIGURE 1. Numerous membrane bounded bodies (asterisks) surround a mast cell (M). The calcium-apatite deposit appears on the elastic matrix (E) and round bodies (arrows).

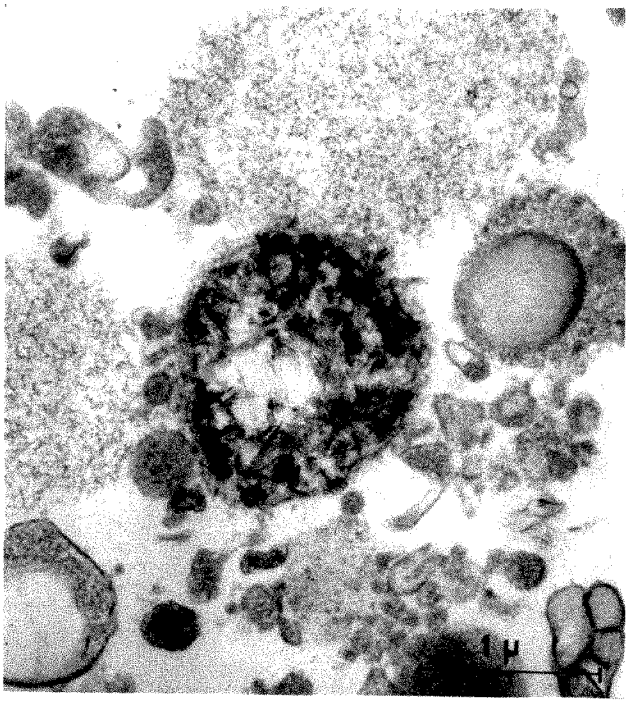

FIGURE 2. Some of the round bodies are membrane bounded and contain needle-shaped crystallites.

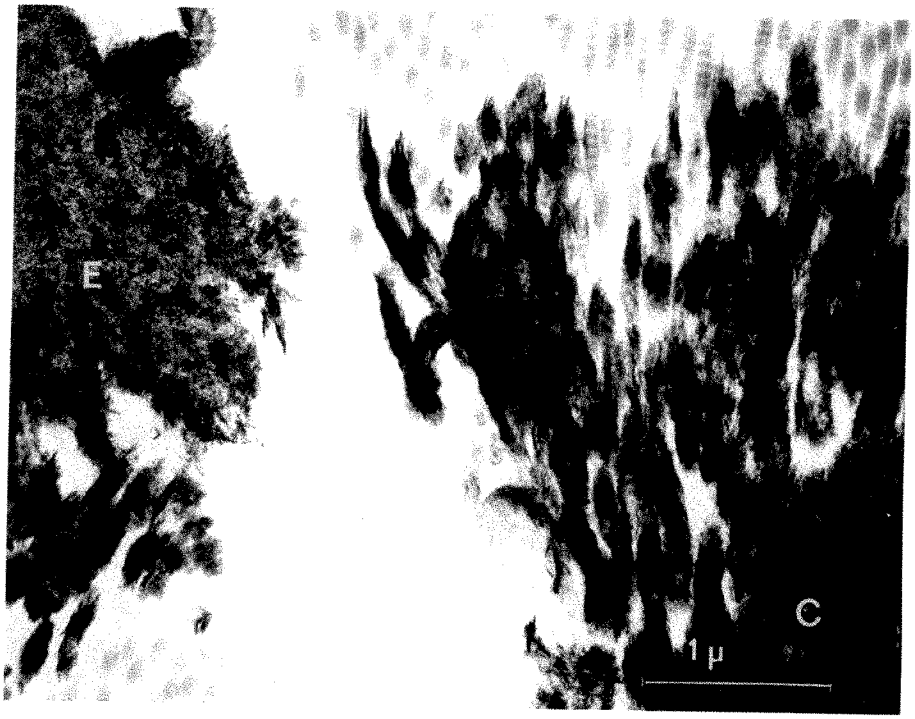

FIGURE 3. The distribution of calcium-apatite crystallites on collagen fibrils (C) and elastic matrix (E) is shown.

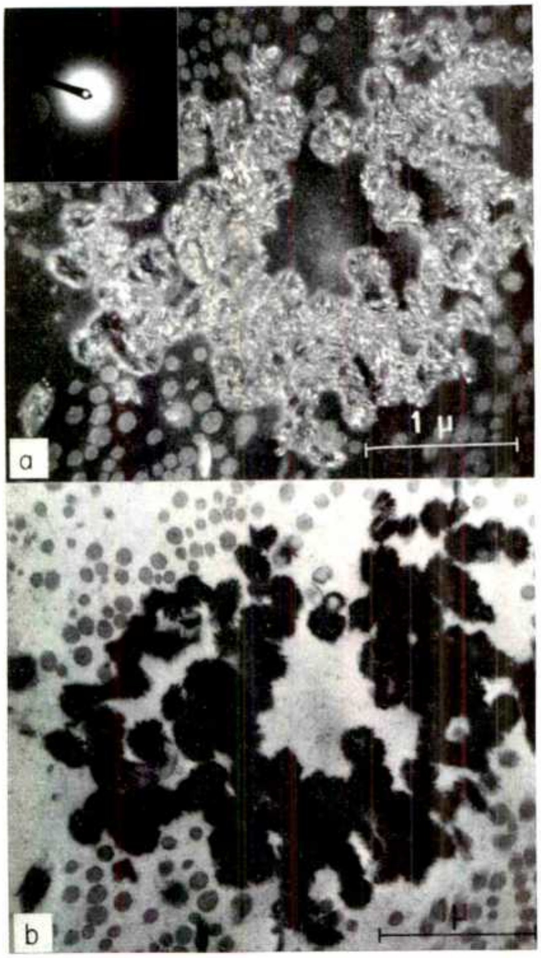

FIGURE 4. Dark-field (a) and bright-field (b) micrographs of calcified collagen fibrils in unstained specimens. The cross-section of the collagen fibrils shows an uneven crystallite distribution. The dark-field micrograph is formed by diffracted electrons as indicated by the position of the objective aperture in the diffraction pattern (inset).

were only located on the matrix and appeared at the periphery with needle-like crystallites while the centre region appeared granular (Figs 3 and 6b). In some instances the deposits in both collagen fibrils and elastic matrix appeared as small foci, while in others, the structures were solidly mineralized. At the end of the third week of culture, large areas of the dermis were occupied by the deposits.

None of the UP skin explants studied before cultivation and none of the cultured skin explants from healthy individuals contained crystalline deposits.

### *Electron diffraction*

Independent of the location, the selected area diffraction patterns of the mineralized deposits showed sharp rings indicating crystallinity (Figs 4a and 5a). The planar distances obtained from the diffraction patterns as described by Johnson & Otkjær Nielsen (1978) are given in Table 1. The planar spacings for calcium hydroxyapatite and calcium fluoroapatite obtained from the file-card index of The

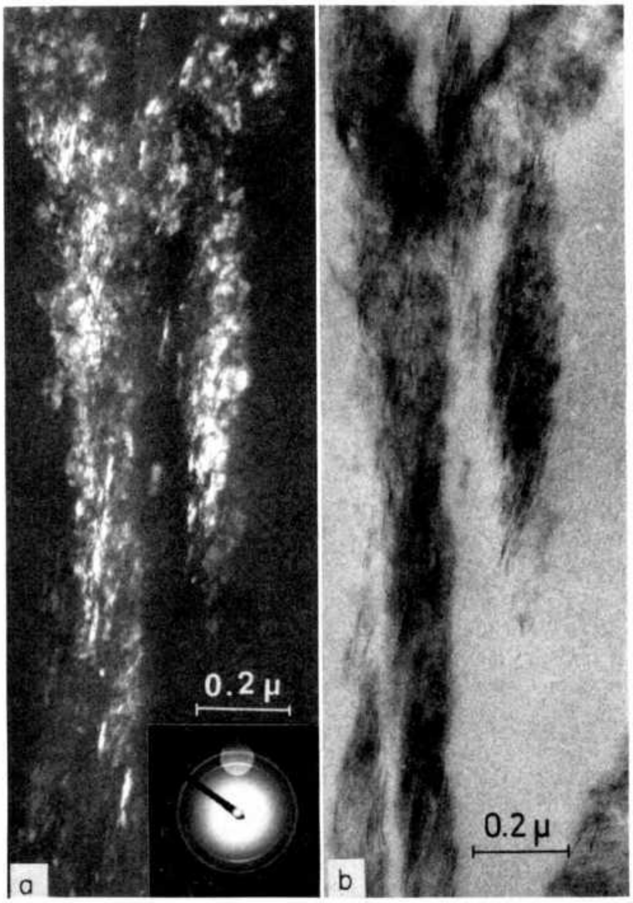

FIGURE 5. The dark-field (a) and bright-field (b) micrographs of longitudinal section of calcified collagen fibrils show that the axis of the needle-shaped crystallites is parallel to the collagen fibril axis. The dark-field micrograph is formed by the intensely textured (0002) diffraction ring (inset).

American Society of Testing and Materials (ASTM) are shown in the same table. The planes were indexed conventionally using the Miller-Bravais four-index notation (Barrett & Massalski, 1966).

The diffraction patterns from the deposits in longitudinally sectioned collagen fibrils showed strong arcing of the diffraction rings indicating a well developed texture (Fig. 5a).

The diffraction pattern from round electron dense bodies without needle structures, collagen fibrils and elastic matrix showed no sharp diffraction rings.

### *Dark-field microscopy*

The use of electrons diffracted from crystalline deposits for imaging makes it possible to study the crystallite distribution. Figures 4–6 show the bright-field and dark-field micrographs from calcified deposits of the connective tissue in unstained sections. The dark-field micrograph showed the crystallites as bright specks on a dark background. The crystallites are evenly distributed throughout the deposit in the elastic matrix (Fig. 6a). The calcium apatite in the deposits occurs as needle shaped crystallites with hexagonal structure and the c-axis as needle axis. The dark-field micrographs of

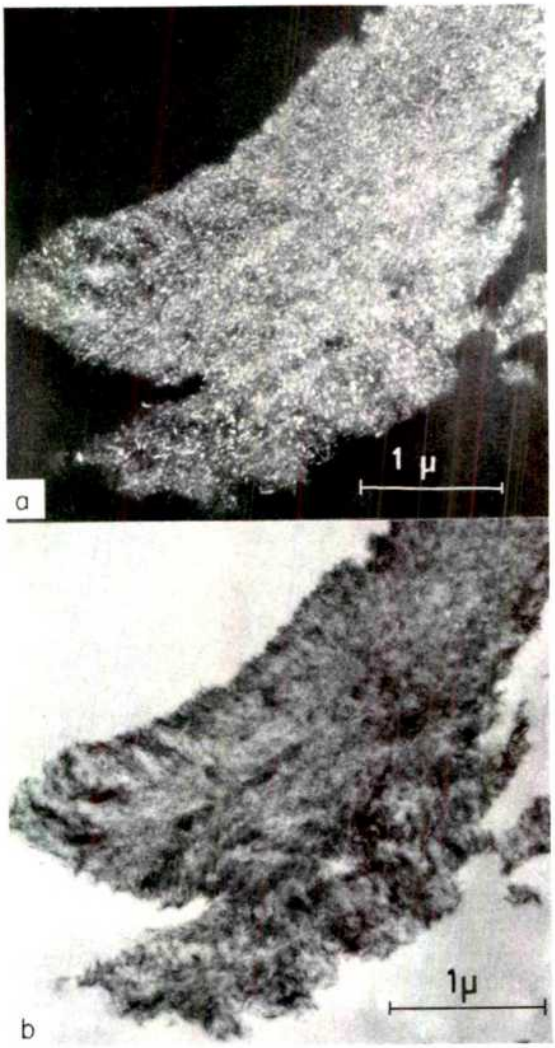

FIGURE 6. The dark-field (a) and bright-field (b) micrographs of calcified elastic matrix in an unstained specimen show the uniform distribution of crystallites.

TABLE 1. Experimental electron diffraction data obtained from calcified deposit in unstained urticaria pigmentosa specimen compared with X-ray diffraction data for calcium fluoroapatite and calcium hydroxyapatite

| | ASTM Data | | Experimental |
|---|---|---|---|
| Miller indices* hkil | Flouoroapatite† d (hkil) nm | Hydroxyapatite‡ d (hkil) nm | Data d (hkil) nm |
| $10\bar{1}0$ | 0·812 | 0·817 | |
| $10\bar{1}1$ | 0·525 | 0·526 | |
| $11\bar{2}0$ | 0·4684 | 0·472 | |
| $20\bar{2}0$ | 0·4055 | 0·407 | |
| $11\bar{2}1$ | 0·3872 | 0·388 | 0·376§ |
| $20\bar{2}1$ | 0·3494 | 0·351 | |
| $00\bar{0}2$ | 0·3442 | 0·344 | 0·343 |
| $10\bar{1}2$ | 0·3167 | 0·317 | 0·316 |
| $21\bar{3}0$ | 0·3067 | 0·308 | |
| $21\bar{3}1$ | 0·2800 | 0·2814 | 0·282 |
| $11\bar{2}2$ | 0·2772 | 0·2778 | 0·276 |
| $30\bar{3}0$ | 0·2702 | 0·2720 | |
| $20\bar{2}2$ | 0·2624 | 0·2631 | 0·264 |
| $30\bar{3}1$ | 0·2517 | 0·2528 | |
| $21\bar{3}2$ | 0·2289 | 0·2296 | |
| $31\bar{4}0$ | 0·2250 | 0·2262 | 0·227 |
| $22\bar{4}1$ | 0·2218 | 0·2228 | |
| $31\bar{4}1$ | 0·2140 | 0·2148 | |
| $30\bar{3}2$ | 0·2128 | 0·2134 | |
| $11\bar{2}3$ | 0·2061 | 0·2065 | 0·207 |
| $40\bar{4}0$ | 0·2028 | 0·2040 | |
| $20\bar{2}3$ | 0·1997 | 0·2000 | |
| $22\bar{4}2$ | 0·1937 | 0·1943 | 0·194 |
| $31\bar{4}2$ | 0·1884 | 0·1890 | |
| 3250 | 0·1862 | 0·1871 | |
| 2133 | 0·1837 | 0·1841 | 0·184 |
| $32\bar{5}1$ | 0·1797 | 0·1806 | |
| $41\bar{5}0$ | 0·1771 | 0·1780 | |
| $40\bar{4}2$, $30\bar{3}3$ | 0·1748 | 0·1754 | |
| $00\bar{0}4$, $41\bar{5}1$ | 0·1722 | 0·1722 | 0·172 |

* The four-index Miller Bravais notation, commonly used for hexagonal crystals. The index i is given by i = −(h+k).

† ASTM card No 15-876. Calcium fluoroapatite has hexagonal crystal structure with $a_0$ = 0·93684 nm and $c_0$ = 0·68841 nm.

‡ ASTM card No 9-432. Calcium hydroxyapatite has hexagonal crystal structure with $a_0$ = 0·9418 nm and $c_0$ = 0·6884 nm.

§ Extremely weak diffraction ring.

cross-sectioned calcified collagen fibrils (Fig. 4a) showed a more irregular distribution of coarse crystallites. The pronounced alignment of needle-shaped crystallites was seen in bright-field as well as dark-field micrographs of longitudinally sectioned collagen fibrils (Figs 5a and 5b). The needles were arranged parallel to the axis of the individual collagen fibril corresponding to the direction in the diffraction patterns connecting the intense (0002) texture arcs used for dark-field imaging.

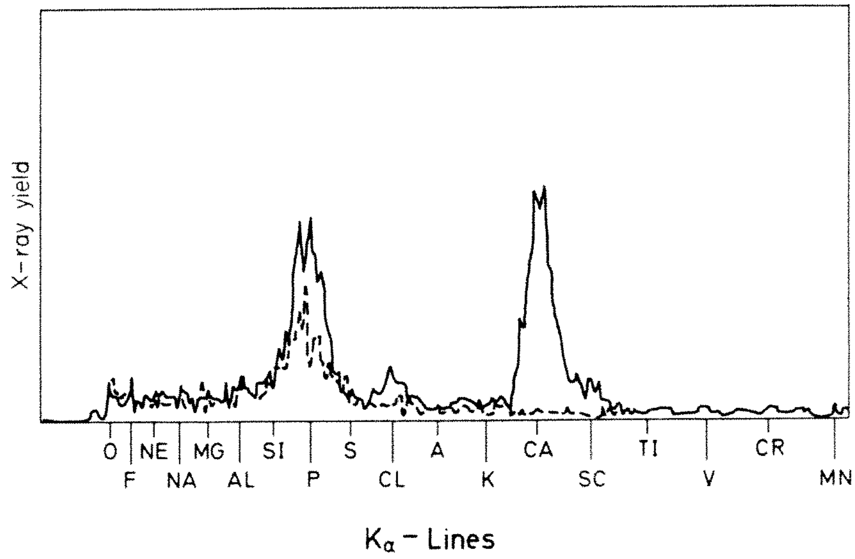

FIGURE 7. The X-ray fluorescence spectrum of calcified collagen fibrils (solid line) shows peaks of calcium, phosphorus and osmium. The peak in the spectrum of uncalcified collagen fibrils (dotted line) originates from osmium used as fixation agent. Ordinate: X-ray yield (arbitrary units). Abscissa: Elements.

### *X-ray microanalysis*

The X-ray fluorescence elemental scan from the deposits of the connective tissue showed peaks originating from calcium at 3·7 keV, and phosphorus and osmium at 2·1 keV (Fig. 7). The spectra obtained from calcified collagen fibrils, elastic matrix and round bodies were identical. The osmium was used for post-fixation of the specimens.

## DISCUSSION

The calcification demonstrated in cultivated UP skin was a significant finding and not an artefact due to the culture medium, fixation, staining or other technical procedures, since the cultured skin of normal individuals studied under identical conditions showed no deposition.

The X-ray microanalysis demonstrated the presence of calcium and probably phosphorus in the deposits of cultured UP skin. The microanalysis was performed on unstained but osmium fixed specimens. The peaks originating from osmium and phosphorus are incidentally close together and cannot be properly resolved, thus rendering the element identification somewhat uncertain (Johnson & Otkjær Nielsen, 1978).

The selected area electron diffraction showed that the deposits have an apatite structure and consisted mainly of calcium hydroxyapatite or possibly calcium fluoroapatite. The accuracy of our meassurements was 1%, and at least 0·5% should be required to allow a distinction between the two apatites (Johnson & Otkjær Nielsen, 1978). The texture of crystallites located on collagen fibres is verified from the arcing of the (0002) diffraction ring (Fig. 5). Correlation between micrographs and diffraction patterns shows that the crystallites, with their needle axis parallel to the collagen fibres, have the c-axis as needle axis. The X-ray diffraction patterns from bone material showed a strong similarity to the electron diffraction from the apatite deposits of cultivated UP skin (Eanes, 1973).

The dark-field microscopy is useful for studies of crystallites and their distribution in calcified connective tissue (Johnson & Otkjær Nielsen, 1978). The dark-field micrographs show the individual crystallites as bright specks on a dark background. Hence, crystallites entirely obscured in the bright-field are visualized in the dark-field micrographs.

The nature of the calcium apatite of cultured UP skin is identical to those found in calcifying pathological skin (Otkjær Nielsen *et al.*, 1977, 1978, 1979) and calcifying hard-tissues (Bonucci, 1971; Anderson, 1973; Eanes, 1973). Hence, the study of hard-tissue calcification may help explain the mechanism of general calcification. The initial mineralization of cartilage and bone takes place within matrix vesicles, originating from cells of the respective tissues (Anderson & Matsuzawa, 1970; Anderson, 1973; Anderson & Reynolds, 1973). The round bodies in the vicinity of mast cells in cultured UP skin morphologically remind us of these matrix vesicles. However, the matrix vesicles have a smaller average diameter (approximately 100 nm) than that of the round bodies (Anderson, 1973; Anderson & Reynolds, 1973) and have never been demonstrated in calcifying soft tissues.

Another possibility is that the round bodies represent disintegrated extracellular mast cell granules. This assumption is supported by the finding that a few round bodies had lamellar structures, which seem to be a specific character of human mast cell granules (Kobayasi, Midtgaard & Asboe-Hansen, 1968). In addition, it has been shown that the mastocalcergy and mastocalciphylaxis phenomena are related to the discharged mast cell granules. Selye (1962) was the first to notice the association between discharged mast cell granules and calcification. The term mastocalciphylaxis was introduced for the condition of experimental calcification produced by a calciphylactic sensitizer (i.e. vitamin D, parathyroid hormones and ferric dextran) followed after a critical time interval by a challenger (i.e. mast cell dischargers). The discharged mast cell granules attract calcium and phosphate and subsequently calcify. Calcification without previous sensitization was termed calcergy. Most calcergens are metallic compounds (e.g. lead acetate). Mastocalcergy is a direct calcification produced by lead acetate and a mast cell discharger. The lead ions are deposited in the mast cell granules which secondarily attract calcium and phosphate (Selye & Gabbiani, 1964; Selye, Gabbiani & Serafimov, 1964).

The results of the electronmicroscopy, electron diffraction and X-ray microanalysis confirm the presence of apatite crystallites in the calciphylactic rat skin lesions (Gabbiani, Badonnel & Baud, 1969). The apatite crystallites of rat skin calciphylaxis were initially deposited in the intercellular compartment of connective tissue as electron dense bodies with a diameter of approximately 100 nm (Gabbiani, Tuchweber & Selye, 1973). The initial skin calcification induced by calciphylaxis appeared within round membrane bounded bodies surrounding dermal cells and subsequently appeared on collagen fibrils (Boivin, 1975). It is an open question whether only the round bodies are responsible for the initial calcification of UP skin explant. It is assumed that the initial calcification continues on collagen fibrils and elastic fibres, because the tissue is supersaturated in regard to apatite crystals (Anderson & Reynolds, 1973). Despite the fact that collagen fibrils are frequently involved in mineralization, they are never the initial site of calcification (Bonucci, 1971).

The present study demonstrates that experimentally induced skin calcifications *in vitro* and *in vivo* have many points of resemblance. The *in vitro* calcification of UP skin is not pathognomonic since uninvolved skin of patients with pseudoxanthoma elasticum has a similar latent liability for calcification which becomes manifest when grown in organ cultures (Hentzer *et al.*, 1977).

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# Buccal mucosa immunofluorescence in coeliac disease and dermatitis herpetiformis

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## SUMMARY

The buccal mucosa in both coeliac disease and dermatitis herpetiformis behaves in the same manner as the skin with regard to immunofluorescence. The simple technique of buccal punch biopsy can be used to give further confirmation of the diagnosis of dermatitis herpetiformis and, especially in patients with an equivocal diagnosis, may prove easier than carrying out repeated skin biopsies.

The cutaneous deposition of IgA is detectable in most patients with dermatitis herpetiformis (DH), but not in coeliac disease (CD) (Seah *et al.*, 1972; Karlsson, Dahl & Marks, 1978). Since oral lesions may occur in both conditions and positive immunofluorescence has been recorded in early buccal lesions in DH (Fraser, Kerr & Donald, 1973), we decided to investigate uninvolved buccal mucosa in two groups of patients—those with DH and those with CD and no evidence of DH.

## PATIENTS, METHODS AND RESULTS

Ten patients with CD and seven with DH (two of whom were known to have a gluten-sensitive enteropathy) were studied. The average age of the CD patients was 34 years (range 18–60), there being eight females and two males, and the average age of the DH patients was 54 (range 16–83), there being five females and two males. Patients with CD had the diagnosis confirmed by jejunal biopsy and each was on a gluten-free diet. Patients with DH had the diagnosis confirmed by the presence of characteristic skin histology and cutaneous fluorescence for IgA. Five patients with DH were on dapsone (the two with an enteropathy also receiving a gluten-free diet) and the other two patients being newly diagnosed were untreated.

A 4 mm punch biopsy was taken from the lateral buccal mucosa in each patient, local anaesthesia being achieved using 2% lignocaine with adrenalin. Each specimen was examined in the standard manner (Lai A Fat, Cormane & van Furth, 1974) for direct IgG, IgM, IgA and C3 fluorescence.

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Nine out of ten patients with CD had negative immunofluorescence findings, the other patient showing only weak and scanty microgranular fluorescence for C3. In two patients, the buccal mucosa was examined for reaction with autologous serum, but this also proved negative. However, all DH patients showed a microgranular pattern for IgA and C3, particularly in papillary tips. In three patients the pattern was weak, but in the other four the changes were as striking as in the skin. There was no correlation between the strength of reaction and treatment with dapsone or presence of enteropathy.

## DISCUSSION

Our immunofluorescence findings for buccal mucosa in CD are similar to those described for the skin (Seah *et al.*, 1972; Karlsson *et al.*, 1978), namely that there are no changes in CD in the absence of DH. The diagnosis of DH depends on the identification of fluorescence for IgA in uninvolved skin (Fry & Seah, 1974) and we have shown the same changes occurring in the uninvolved buccal mucosa of such patients.

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# Acquired ichthyosis and impaired dermal lipogenesis in Hodgkin's disease

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## SUMMARY

Epidermal lipid biosynthesis was normal in patients with mild ichthyosis due to Hodgkin's disease, but greatly reduced in one patient with severe ichthyosis. Dermal (sebaceous) lipid synthesis was decreased in all patients with Hodgkin's disease, whether or not they had ichthyosis, and was greatly reduced in the patient with severe ichthyosis. Neither the mechanism nor the possible relationship between the dermal and epidermal changes is understood.

An acquired ichthyosis which closely resembles the autosomal dominant variety may develop in the course of Hodgkin's disease. Many disorders of keratinization are accompanied by changes in epidermal lipid biosynthesis (Ziboh & Hsia, 1972; Prottey & Hartop, 1973; Cooper, McGrath & Shuster, 1976a; Cooper, McGibbon & Shuster, 1978; Dykes & Marks, 1978; Cooper *et al.*, 1978); conversely, drugs which modify lipid metabolism can cause acquired ichthyosis (Yardley, 1969). We therefore examined the possibility that a change in lipid biosynthesis could be responsible for the acquired ichthyosis of Hodgkin's disease.

## PATIENTS AND METHODS

We studied seven patients with acquired ichthyosis due either to Hodgkin's disease or to related lymphomas. One of the patients with stage 2B disease was referred to the skin clinic with severe ichthyosis; the others had mild ichthyosis and were detected at a clinic for the treatment of Hodgkin's lymphoma. The clinical diagnosis was confirmed histologically by hyperorthokeratosis and absence of the granular layer in biopsies of skin from the region of the shoulder blade. Five patients with Hodgkin's disease but with clinically and histologically normal skin were also studied. No patient in the study was receiving radiotherapy or chemotherapy; the intervals between termination of the last course of therapy and commencement of the study ranged from 1 month to 9 years, with a mean interval of 2·4 years. Both groups were comparable with respect to stage of disease and apparent

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nutritional status. For comparison a group consisting of twelve patients with minor skin disorders (e.g. warts or mild acne) were studied; they were matched for sex and age (to within 10 years) with the patients with Hodgkin's disease.

Dermal and epidermal lipid biosynthesis was measured by [$^{14}$C] glucose incorporation into lipids in 4 mm punch biopsies of shoulder blade skin, as previously described (Cooper *et al.*, 1976a, Cooper, McGrath & Shuster, 1976b). In the patients with Hodgkin's disease sebum excretion rate on the forehead (Cunliffe & Shuster, 1969), fasting plasma cholesterol and triglycerides, and the fatty acid profile of plasma lecithin were also measured.

## RESULTS

Epidermal lipogenesis as $^{14}$C incorporation into total lipids was normal in the patients with Hodgkin's disease who had mild or no ichthyosis. In the one patient with severe ichthyosis, however, epidermal lipid labelling was almost undetectable (Fig. 1).

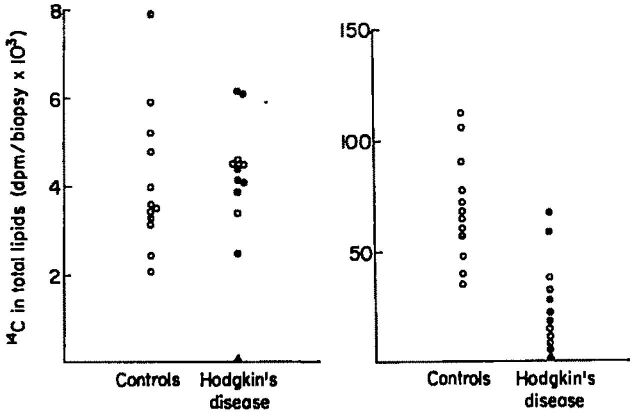

Fig.1 Fig.2

FIGURES 1 AND 2. $^{14}$C incorporation into total epidermal lipids (Fig. 1) and total dermal lipids (Fig. 2) in skin biopsies from normal controls and from patients with Hodgkin's disease having clinically normal skin (○), mild acquired ichthyosis (●) or severe acquired ichthyosis (▲). Results are expressed as dpm per 4 mm punch biopsy.

Incorporation of $^{14}$C into the different lipid classes of epidermis is shown in Table 1. No significant differences were seen between the control group and the patients with Hodgkin's disease with or without mild ichthyosis. In the patient with severe ichthyosis, $^{14}$C incorporation into total lipids was too low to permit further analysis.

Dermal lipogenesis as $^{14}$C incorporation into total lipids was low in the patients with Hodgkin's disease compared with normal controls (Fig. 2). This reduction in dermal activity occurred equally in patients with and without ichthyosis but was particularly marked in the patient with severe ichthyosis. To exclude the possibility of a technical error a second biopsy was taken from this patient several weeks later and it gave almost identically low rates both of dermal and epidermal lipogenesis. Polar lipids, neutral glycerides, free fatty acids, and the fraction containing wax and sterol esters were all affected (Table 1). By contrast, labelling of free sterols was increased ($P < 0{\cdot}05$) (Table 1). Squalene labelling appeared to be somewhat reduced, but because of the high variability which is characteristic of this lipid class the difference was not significant (Table 1).

TABLE 1. Incorporation of $^{14}$C into lipid classes of epidermis and dermis of skin biopsies from normal controls and patients with Hodgkin's disease (with or without acquired ichthyosis). Results are expressed as dpm per 4 mm punch biopsy (mean ± s.e. mean), and $P$ values are for Student's '$t$'-test (NS = not significant at $P = 0.05$)

| | Epidermis | | | Dermis | | |
|---|---|---|---|---|---|---|
| Lipid class | Controls (n = 12) | Hodgkin's (n = 11) | $P$ | Controls (n = 12) | Hodgkin's (n = 12) | $P$ |
| Polar lipids | 2382 ± 234 | 2300 ± 140 | NS | 5925 ± 494 | 1836 ± 296 | <0·001 |
| Monoglycerides | 210 ± 57 | 227 ± 18 | NS | 668 ± 72 | 437 ± 60 | <0·025 |
| Free fatty acids | 326 ± 133 | 257 ± 58 | NS | 2881 ± 351 | 754 ± 171 | <0·001 |
| Free sterols | 308 ± 36 | 383 ± 32 | NS | 305 ± 43 | 728 ± 176 | <0·05 |
| 1,2-Diglycerides | 254 ± 34 | 349 ± 63 | NS | 1981 ± 289 | 1012 ± 265 | <0·05 |
| 1,3-Diglycerides + fatty alcohols | 113 ± 12 | 133 ± 28 | NS | 1198 ± 133 | 669 ± 268 | NS |
| Triglycerides + glyceryl ether lipids | 353 ± 44 | 459 ± 65 | NS | 38637 ± 4117 | 13777 ± 3433 | <0·001 |
| Wax diesters | 31 ± 8 | 29 ± 8 | NS | 813 ± 179 | 410 ± 102 | (<0·1) |
| Wax monoesters + sterol esters | 27 ± 7 | 20 ± 5 | NS | 6301 ± 736 | 1998 ± 578 | <0·001 |
| Squalene | 40 ± 7 | 41 ± 5 | NS | 7797 ± 1666 | 4711 ± 1501 | NS |

TABLE 2. Sebum excretion rate (forehead) in patients with Hodgkin's disease compared with accumulated data for normal controls (mean ± s.e. mean; $P$ values are for Student's '$t$'-test—NS = not significant at $P = 0·05$)

| | Sebum excretion rate ($\mu$g/cm$^2$/min) | |
|---|---|---|
| | Male | Female |
| Hodgkin's disease | 1·03 ± 0·19 (n = 7) | 0·47 ± 0·13 (n = 5) |
| Normal controls (20–69 years, male; 20–59 years, female) | 1·23 ± 0·07 (n = 54) | 0·61 ± 0·05 (n = 72) |
| $P$ | NS | NS |

TABLE 3. Plasma lipids in patients with Hodgkin's disease, compared with normal ranges. Fatty acid abbreviations are as follows: 18:2, linoleic acid; 20:4, arachidonic acid; 20:3 $\omega$9, $\omega$9-eicosatrienoic acid

| | Plasma cholesterol (mmol/l) | Plasma triglycerides (mmol/l) | Plasma lecithin fatty acids (% of total) | | |
|---|---|---|---|---|---|
| | | | 18:2 | 20:4 | 20:3 $\omega$9 |
| Hodgkin's—severe ichthyosis | 12·0 | 3·2 | 12·1 | 2·0 | 1·6 |
| Hodgkin's—mild ichthyosis (mean ± s.e. mean, n = 5) | 6·2 ± 0·2 | 1·2 ± 0·1 | 22·0 ± 1·5 | 8·5 ± 1·1 | Trace |
| Hodgkin's—normal skin (mean ± s.e. mean, n = 5) | 6·3 ± 0·3 | 1·1 ± 0·3 | 25·2 ± 1·1 | 8·3 ± 0·7 | Trace |
| Normal range | 3·6–7·3 | 0·5–1·8 | 17–25 | 8–10 | Trace |

By contrast with dermal lipogenesis sebum excretion rate in patients with Hodgkin's disease was slightly lower than in normal individuals with minimal skin disorders, but the differences were not statistically significant (Table 2).

Plasma lipid analyses are shown in Table 3. In the patient with severe ichthyosis, plasma cholesterol and triglyceride levels were slightly increased. Plasma lecithin fatty acids showed a decrease in linoleic and arachidonic acids (18:2 and 20:4), together with the appearance of a small amount of the $\omega 9$ isomer of eicosatrienoic acid (20:3 $\omega 9$). In the other patients with Hodgkin's disease, plasma cholesterol and triglycerides were within the normal range, and the plasma lecithin fatty acid profile was normal.

## DISCUSSION

The present results suggest that epidermal lipid biosynthesis is normal in patients with mild acquired ichthyosis due to Hodgkin's disease. This contrasts with autosomal dominant ichthyosis vulgaris, in which epidermal lipogenesis is increased (Cooper *et al.* 1978; Dykes & Marks, 1978) and suggests that the mechanism of the two conditions is different even though they are clinically and histologically similar. Epidermal incorporation of $^{14}$C acetate into lipids was also found to be normal in patients with mild ichthyosis associated with intestinal malabsorption (Dykes, Marks, & Smith, 1978).

In the one patient with severe acquired ichthyosis, epidermal lipid biosynthesis was very low. Drugs which inhibit lipid synthesis can cause acquired ichthyosis (Yardley, 1969), and it seems possible that in this patient the severity of the ichthyosis was increased by the low level of lipogenic activity. The mechanism of the impaired epidermal lipogenesis is not clear and the high plasma cholesterol and triglyceride levels in this patient do not point to a generalized block in lipid synthesis in other tissues such as occurs with drug-induced ichthyosis. However the low levels of linoleic and arachidonic acids in plasma lecithin, combined with the appearance of $\omega 9$-eicosatrienoic acid, are suggestive of essential fatty acid deficiency (Alfin-Slater & Aftergood, 1968). As this patient had been anorexic for some months, and had lost 3 stones in weight, it is possible that she had suffered from mild essential fatty acid deficiency and was recovering at the time when the plasma sample was taken. Essential fatty acid deficiency causes scaling of the skin, which is reversible by treatment with linoleic or arachidonic acid. It is unlikely that essential fatty acid deficiency contributed to the ichthyosis in this case, because topical treatment neither with sunflower seed oil which is rich in linoleic acid nor with cod liver oil which is rich in arachidonic acid, for 3 weeks produced any clinical improvement. Moreover, the decrease in epidermal lipogenesis contrasts with the great increase in cutaneous lipid synthesis found in essential fatty acid deficiency (Ziboh & Hsia, 1972). Essential fatty acid levels in the other patients with acquired ichthyosis were normal, which further suggests that the ichthyosis in Hodgkin's disease is not associated with essential fatty acid deficiency.

An unexpected finding in this study was the decrease in dermal lipogenesis shown by all patients with Hodgkin's disease. As both epidermal lipogenesis and plasma lipid levels were normal, the change in lipid metabolism may be specific to the dermis. Lipid synthesis in the dermis is confined almost entirely to the sebaceous glands (Cooper *et al.*, 1976b; Summerly, 1974) and since sebaceous secretion is holocrine the rate of dermal lipogenesis and sebum excretion correlate well (Cooper *et al.*, 1976a, b). The dissociation between these two indices of sebaceous activity appears to be unique to Hodgkin's disease. There are two possible explanations for this disturbance of sebaceous lipogenesis in Hodgkin's disease: either $^{14}$C glucose incorporation does not accurately represent changes in dermal lipogenesis (e.g. from a distortion of pool size), or a greater proportion of sebaceous lipid is derived from sources other than glucose, e.g. from amino acids (Wheatley, 1974) or from plasma.

The question which arises is whether the changes we have demonstrated in dermal lipogenesis may be the cause of the epidermal ichthyotic defect which provoked this study. This explanation would be in keeping with the possibility that sebaceous glands may provide substrate for the epidermis (Shuster, 1976) and merits direct experimental study.

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# A method for the evaluation of the local antiandrogenic action of 5α-reductase inhibitors on human skin

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## SUMMARY

A method has been developed for the first time that allows the evaluation of the effect of therapeutic concentrations of 5α-reductase inhibitors in human skin, without applying radioactivity to the skin. Moreover, the method makes it possible to determine whether inhibition is found only at the application site or also in other parts of the skin.

During the last 10 years, the skin has been recognized as a major site for androgen metabolism, comparable to the prostrate gland. This finding was preceded by two discoveries with regard to testosterone metabolism: first, dihydrotestosterone rather than testosterone is the most potent androgen in certain tissues; second, dihydrotestosterone is formed mostly in the target cells *per se* by the 5α reduction of testosterone (Price, 1975).

It is generally believed that 5α-dihydrotestosterone may be responsible for stimulation of the sebaceous gland (Takayasu & Adachi, 1972) and in acne the evidence for increased local formation of dihydrotestosterone has been documented (Sansone & Reisner, 1971). It has been shown that 5α-reductase inhibitors provoke a local anti-androgenic effect in laboratory animals (Voigt & Hsia, 1973; Vermorken, Goos & Roelofs, 1980b). No method was available, however, to establish the local effect of therapeutic concentrations of these inhibitors on human skin.

The hair follicle forms an ideal biopsy tissue for the study of testosterone metabolism in the human skin since it is easily obtained without any harm to the patient and since it retains its metabolic activity for a few hours after isolation.

In this study hair follicles have been used in order to evaluate the efficiency of the 5α-reductase inhibitor progesterone on the human skin. The method also enables one to distinguish between a local and a systemic effect of the inhibitor.

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## MATERIALS AND METHODS

[4-$^{14}$C] Testosterone (specific activity 51 mCi/mmol), [4-$^{14}$C] androstenedione (specific activity 57 mCi/mmol) and [4-$^{14}$C] dehydroepiandrosterone (specific activity 55 mCi/mmol), were obtained from the Radiochemical Centre, Amersham, and were checked chromatographically for radiochemical purity before each experiment. Unlabelled steroids were purchased from Sigma Chemical Company. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP were obtained from Boehringer Mannheim.

Penicillin G was obtained from Gist Brocades, The Netherlands. For thin layer chromatography silica gel plates $F60_{254}$, obtained from Merck, were employed. Kodak X-Ray XR-2 films were used for autoradiography. A solution of 3 g of PPO (2,5-diphenyloxazole) and 0·2 g of POPOP (2,2'-*p*-phenylenbis (5-phenyloxazole)), both obtained from Merck, in 1 l of toluene was used as scintillation fluid.

### *Tissue preparation*

Unless otherwise indicated, hair follicles were plucked from the frontal and occipital areas of the scalp of normal human males and females and only those with a visible bulb and sheath were used. The hair follicles were cut off just above the sheath and immediately immersed in incubation medium.

### *Progesterone application*

In the experiments where the effect of topically applied progesterone on the metabolism of testosterone was investigated, 4 ml of a progesterone solution (0·5% solution in 50% ethanol) were applied to a small area of the scalp (± 10 cm$^2$). At several times after application (depending on the type of experiment) hair follicles were plucked from the scalp and the metabolism of radioactive testosterone was measured.

### *Incubation*

The radioactive steroid (0·330 $\mu$Ci) was added to the incubation mixture in ethanol (less than 2% (v/v) ethanol; the addition of this amount of ethanol had no influence on the enzyme activity).

The incubation mixture consisted of 50 mM Tris-HCl buffer, pH 7·5, containing 0·2 M sucrose, 100 units of penicillin G/ml and a NADPH generating system (1 mM NADP, 20 mM glucose-6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase/ml and 0·5 mM $MgCl_2$). The tissue was incubated at 37°C for 4 h, under air. Unless otherwise indicated the concentration of progesterone added *in vitro* was 100 times that of radioactive testosterone.

### *Isolation of radiometabolites*

The incubation mixture was extracted three times with an equal volume of ethylacetate. The organic phases were combined and evaporated to dryness under nitrogen. The steroids in the dry residue were redissolved in 10 $\mu$l ethanol, applied to thin layer plates and chromatographed with unlabelled markers. A system of chloroform–methanol (98·25–1·75 (v/v)) was used for the separation of testosterone metabolites and a system of chloroform–ethanol (38–2 (v/v)) was used for the separation of androstenedione and dehydroepiandrosterone metabolites.

The unlabelled steroids were visualized by spraying the plates with a 1% anisaldehyde solution in sulphuric acid–acetic acid (1–50 (v/v)), after which the plates were heated for 5 min at 90°C.

The spectrum of metabolites was examined autoradiographically by exposure of the plates to X-ray film. For quantification of the results the areas of radioactivity were scraped off the plates and transferred to counting vials, which contained 1 ml of methanol. For counting 10 ml of scintillation fluid was added.

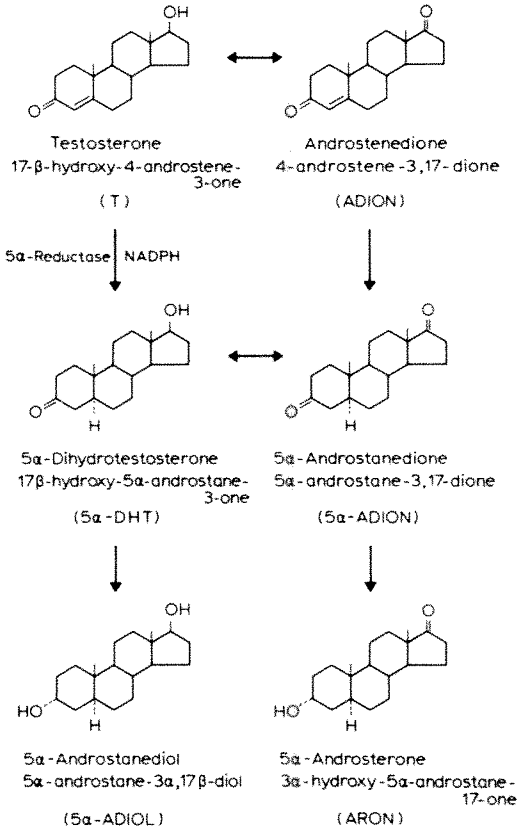

FIGURE 1. Metabolism of testosterone in the human skin.

## RESULTS

When isolated male or female human scalp hair follicles are incubated with testosterone, five metabolites are formed (see Fig. 1). In Fig. 2b and b′ the result of such an incubation with radioactive testosterone is shown. Addition of progesterone, the inhibitor of $5\alpha$-reductase to the incubation mixture, in a concentration of 100 times that of testosterone results in the complete inhibition of the formation of all the $5\alpha$ reduced metabolites (Fig. 2c and c′).

The results of an experiment using different concentrations of progesterone are shown in Fig. 3.

When isolated male or female human scalp hair follicles are incubated with androstenedione, three major metabolites are formed namely, testosterone, $5\alpha$-dihydrotestosterone and $5\alpha$-androstanedione (Fig. 4b and b′). Addition of progesterone results in a strong inhibition of the formation of the $5\alpha$ metabolites. Incubation of hair follicles with dehydroepiandrosterone results in the formation of androstenediol and $7\alpha$-hydroxydehydroepiandrosterone (Fig. 5). A minor quantity of androstenedione is also formed (Vermorken *et al.*, 1979). Addition of progesterone does not result in inhibition of the formation of any of the metabolites (see Fig. 5).

Figure 6 shows that after topical application of 20 mg of progesterone, in an alcohol/water mixture, on 10 $cm^2$ of the surface of the scalp, inhibition of $5\alpha$-reductase is indeed found. It is evident that the inhibitory activity is lost after 48 h.

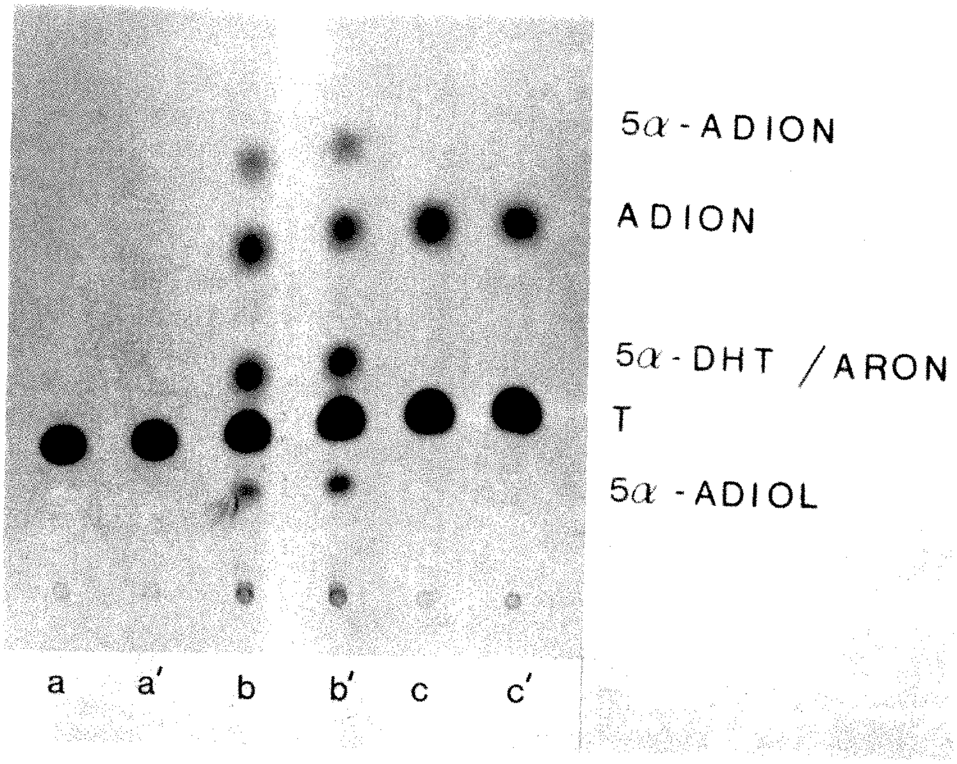

FIGURE 2. Autoradiograph of a chromatogram with the metabolites extracted after incubation of radioactive testosterone: (a, a′) without hair follicles; (b, b′) with ten hair follicles; (c, c′) with ten hair follicles in the presence of progesterone (the volume of the incubation mixture was 200 μl).

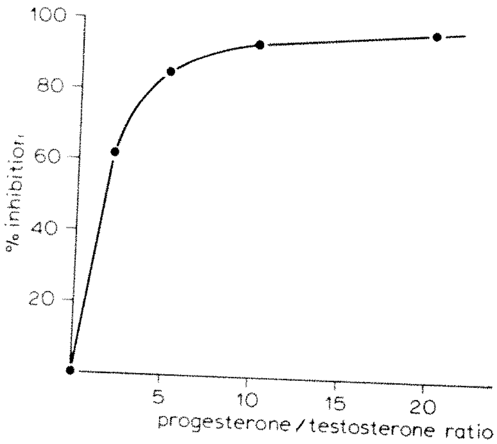

FIGURE 3. Effect of different progesterone concentrations on the 5α-reductase activity.

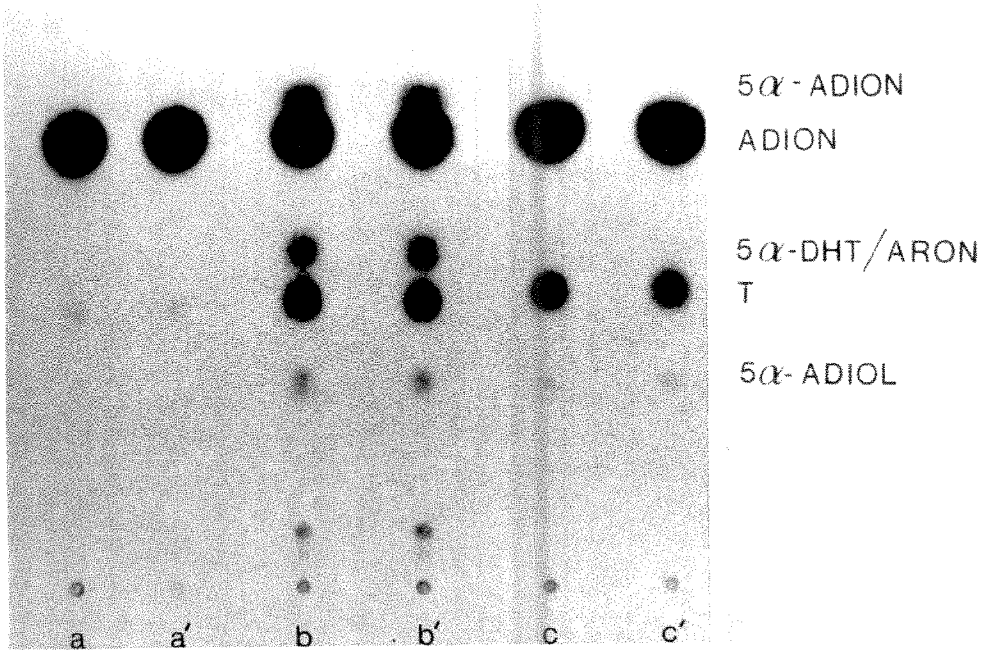

FIGURE 4. Autoradiograph of a chromatogram with the metabolites extracted after incubation of radioactive androstenedione in 200 μl incubation mixture: (a, a′) without hair follicles; (b, b′) with ten hair follicles; (c, c′) with ten hair follicles in the presence of progesterone.

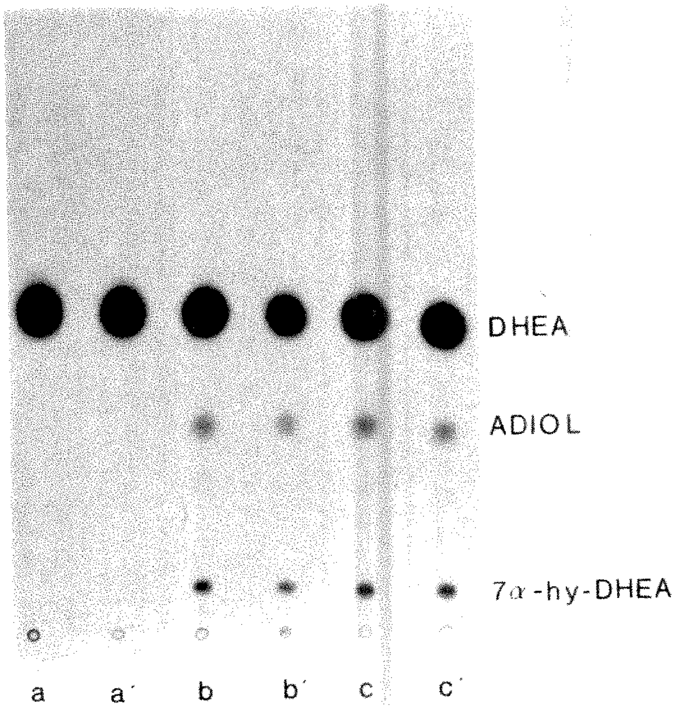

FIGURE 5. Autoradiograph of a chromatogram with the metabolites extracted after incubation of radioactive dehydroepiandrosterone in 200 μl incubation mixture: (a, a′) without hair follicles; (b, b′) with ten hair follicles; (c, c′) with ten hair follicles in the presence of progesterone. (DHEA = dehydroepiandrosterone = 3β-hydroxy-5-androstene-17-one; ADIOL = androstenediol = 5 androstene-3β,17β-diol).

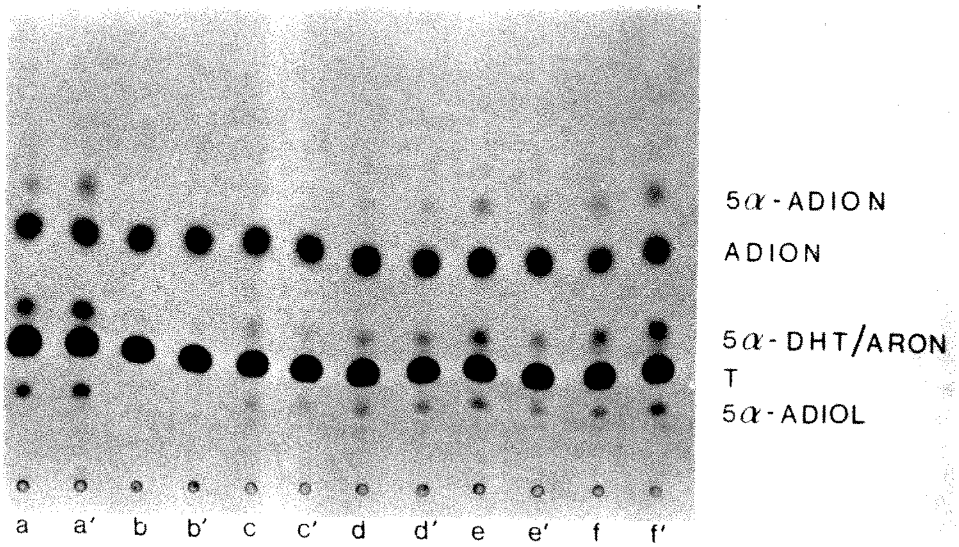

FIGURE 6. Autoradiograph of a chromatogram with the metabolites extracted after incubation of radioactive testosterone with ten hair follicles: (a, a′) before; (b, b′) 10 min; (c, c′) 4 h; (d, d′) 12 h; (e, e′) 24 h; (f, f′) 48 h after application of progesterone on the scalp skin (the volume of the incubation mixture was 50 μl).

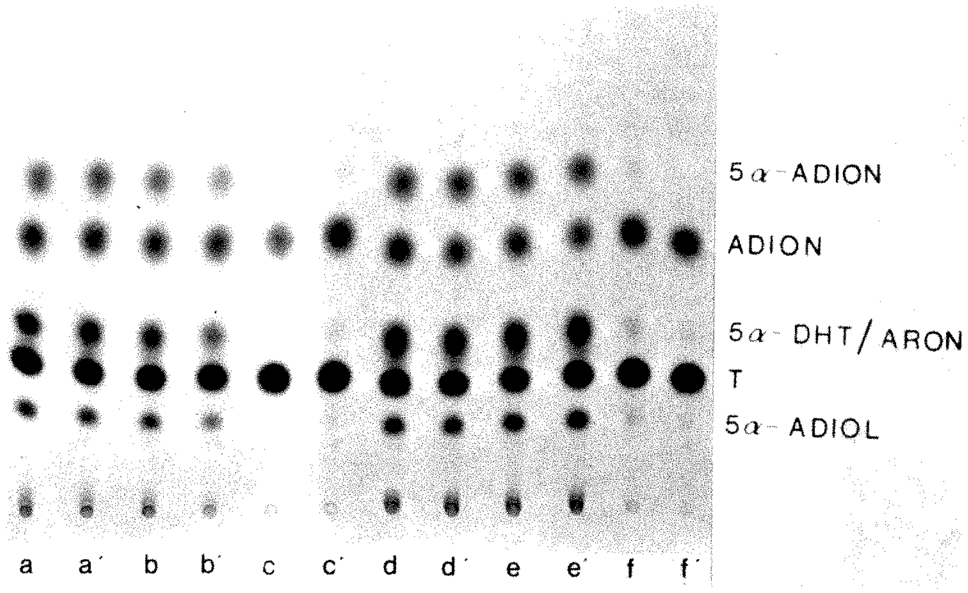

FIGURE 7. Autoradiograph of a chromatogram with the metabolites extracted after incubation of radioactive testosterone: (a, a′) with ten hair follicles isolated before progesterone application; (b, b′) with ten hair follicles isolated 2 min after progesterone application from the application site; (c, c′) with ten hair follicles isolated 4 h after progesterone application from the application site; (d, d′) with ten hair follicles isolated before progesterone application from the control site; (e, e′) with ten hair follicles isolated 2 min after progesterone application from the control site; (f, f′) with ten hair follicles isolated 4 h after progesterone application from the control site (the volume of the incubation mixture was 50 μl).

Figure 7 shows that when the left side of the scalp is treated with progesterone, inhibition of $5\alpha$-reductase is also found in hair follicles on the right side of the scalp. This phenomenon was found in most but not in all individuals tested.

## DISCUSSION

Our results have shown that in males and females progesterone, even at very low concentrations, inhibits $5\alpha$-reductase very strongly. This is in agreement with results of experiments on the hamster flank organ where the ratio between progesterone and testosterone necessary completely to inhibit testosterone action is about 10 (Vermorken, Goos & Roelofs, unpublished results). The fact that formation of $5\alpha$ metabolites from androstenedione is also inhibited is important in women where levels of circulating testosterone are low.

It has recently been claimed that dehydroepiandrosterone may be more sebotrophic than dihydrotestosterone (Cooper *et al.*, 1979). Our results show that progesterone, an inhibitor of sebum secretion at least in women (Simpson *et al.*, 1979), does not influence dehydroepiandrosterone metabolism. Therefore at least in women dihydrotestosterone may be regarded as an important physiological sebotrophic factor. After topical application of progesterone on the skin, formation of $5\alpha$-dihydrotesterone is inhibited. The inhibition is gradually lost over a period of about 48 h. Depending upon the degree of inhibition required, application should therefore be repeated every 12–24 h.

The fact that topical application of progesterone to one side of the scalp also results in inhibition of $5\alpha$-reductase at the contralateral side of the scalp demonstrates that at least in the region of the scalp skin the inhibitory activity of progesterone, at the concentration used, is not confined to the application site. Whether this is due to diffusion in the scalp skin or to a systemic effect has to be further investigated.

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*British Journal of Dermatology* (1980) **102**, 703.

# Effects of hydrocortisone on the adrenaline–adenylate cyclase system of the skin

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## SUMMARY

Using pig skin slices, we investigated the effects of hydrocortisone on the adenylate cyclase system of the skin. In short-term experiments, hydrocortisone, when added singly or in combination with other stimulators of adenylate cyclase in the skin (adrenaline or histamine), had no effect on cyclic AMP accumulation. However, when skin slices were incubated with hydrocortisone for more than 6 h, the response to adrenaline differed, with a greater accumulation of cyclic AMP in the hydrocortisone-treated skin. This effect was seen at a concentration of more than 1 $\mu$M hydrocortisone and was most marked 48 h later, while responses to adrenaline in control skin gradually decreased and remained low.

Histamine, which is another stimulator of adenylate cyclase of the skin, did not cause a greater cyclic AMP accumulation in response to this hydrocortisone treatment. There was no significant difference in either low Km or high Km cyclic AMP phosphodiesterase activities as a result of this hydrocortisone treatment.

Hydrocortisone seems to act by protecting the adrenaline–adenylate cyclase system of the skin.

Glucocorticoids and cyclic AMP induce similar changes in epidermis (Voorhees, Marcelo & Duell, 1975). For example, glucocorticoids inhibit cell proliferation (Fisher & Maibach, 1971; Hennings & Elgjo, 1971; Fisher, Maibach & Trancik, 1978) and stimulate differentiation of epidermis (Sugimoto *et al.*, 1974), and these effects can also be demonstrated by treating epidermis with cyclic AMP (Marks & Rebien, 1972; Flaxman & Harper, 1975b) or dibutyryl cyclic AMP (Voorhees, Duell & Kelsey, 1972; Flaxman & Harper, 1975b; Delescluse, Fukuyama & Epstein, 1976), the cyclic AMP derivative which is more resistant to cyclic AMP-phosphodiesterase (Swislocki, 1970).

Despite these similarities in their functions, it has been suggested that these two compounds work independently (Voorhees *et al.*, 1975), and there is scanty evidence on their interaction in epidermis. In this communication, we report the effect of hydrocortisone on the adrenaline-adenylate cyclase

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system of the skin. It was shown that responses of the control skin to adrenaline gradually decreased during long-term incubation, whereas those of the hydrocortisone-treated skin were well preserved. Since the adrenaline–adenylate cyclase system has been postulated to be one of the most important regulatory systems of epidermal cell proliferation (Powell, Duell & Voorhees, 1971; Voorhees *et al.*, 1972), this effect of hydrocortisone might be significantly concerned with the kinetics of epidermal cell proliferation.

Many reports have been published on the existence of the adrenaline- (or β-adrenergic)-adenylate cyclase system of the skin (Powell *et al.*, 1971; Brønstad, Elgjo & Øye, 1971; Mui, Hsia & Halprin, 1975; Yoshikawa *et al.*, 1975b).

## MATERIALS AND METHODS

Domestic pigs weighing about 15 kg were anaesthetized with Nembutal (Dainippon, Osaka, Japan) intraperitoneally (dose 30 mg/kg body weight), as previously described (Iizuka *et al.*, 1978a). Fifteen minutes after the anaesthesia, skin slices from the backs of the pigs were taken with a Castroviejo keratotome adjusted to a 0·3 mm setting (Iizuka *et al.*, 1976). The skin thus obtained was predominantly epidermis (80–90%) with a little dermal contamination. The skin slice was cut into 5 × 5 mm squares, washed three times and floated, with their keratin layers uppermost, in Hank's Medium.

In short-term experiments (Fig. 1), the skin squares were immediately preincubated at 37°C for 15 min to standardize the cyclic AMP level (Yoshikawa *et al.*, 1975a), the method of incubation being as previously described (Iizuka *et al.*, 1976). After preincubation, two of the skin squares were randomly selected and floated with their keratin layers uppermost in Hank's medium containing different concentrations of the drugs and chemicals to be tested. After incubation at 37°C in a water bath for 5 min, they were quickly frozen between two plates of dry ice. In long-term experiments (Tables 1 and 2; Figs 2 and 3), the skin squares were floated in 10 ml of Eagle's M.E.M. with added antibiotics (0·1 mg/ml streptomycin, 100 units/ml penicillin) and various compounds to be tested, and incubated at 37°C in an atmosphere of 5% $CO_2$ in air. After an appropriate time, the skin squares were washed once and preincubated for 15 min in Hank's medium and then incubated for cyclic AMP accumula-

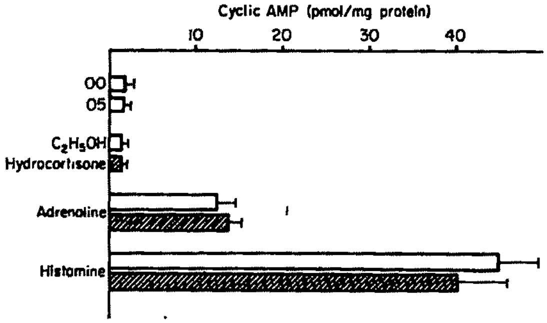

FIGURE 1. The effect of hydrocortisone (short-term experiments). Skin squares were used immediately without the incubation in Eagle's medium. After pre-incubation in Hank's medium for 15 min, incubation was for 5 min at 37°C. No phosphodiesterase inhibitor was added to the media. 00 indicates the cyclic AMP level immediately after the pre-incubation in Hank's medium without adding any chemicals. The concentration of hydrocortisone was 100 μM, and of ethanol 0·5% (v/v). As the control (open column) only ethanol was added. The hatched column shows the cyclic AMP level of the skin treated with hydrocortisone plus ethanol. The concentrations of adrenaline and histamine were 50 μM and 1 mM respectively. Results are the means of five experiments ± s.e.

TABLE 1. Effects of long-term hydrocortisone treatment. Skin squares were incubated in Eagle's M.E.M. for 48 h with and without 100 μM hydrocortisone. After 48 h incubation skin squares were pre-incubated in Hank's medium and then incubated with shown chemicals as described in Materials and Methods section. Results are means ± s.e.

| Agent | Concentration | No. of pairs | Control (pmol/mg protein) | Hydrocortisone treated (pmol/mg protein) |
|---|---|---|---|---|
| None | — | 8 | 1·2 ± 0·3 | 1·1 ± 0·3 |
| Adrenaline | 50 μM | 8 | 3·6 ± 0·5* | 16·8 ± 3·9† |
| Histamine | 1 mM | 8 | 39·5 ± 6·1 | 33·3 ± 8·4 |
| Hydrocortisone | 100 μM | 2 | 1·3 | n.d. |

* $P < 0{\cdot}001$ compared with the initial response (Fig. 1).
† $P < 0{\cdot}02$ compared with control.
n.d.: not done.

TABLE 2. Phosphodiesterase activities in control and hydrocortisone-treated skin. Skin squares were incubated in Eagle's M.E.M. for the shown time with and without 100 μM hydrocortisone. After the incubation, two skin squares were pre-incubated for 15 min in Hank's medium and then frozen between two plates of dry ice. The substrate levels for the low and high Km enzyme assays were 1·2 μM and 103 μM respectively. Results are the means ± s.e. (n = 6)

| Time (h) | Low Km (pmol/min/mg protein) | | High Km (pmol/min/mg protein) | |
|---|---|---|---|---|
| 0 | 19·7 ± 4·7 | | 220 ± 52 | |
| | Control | Hydrocortisone | Control | Hydrocortisone |
| 24 | 20·8 ± 5·5 | 19·3 ± 5·9 | 254 ± 66 | 212 ± 65 |
| 48 | 15·3 ± 4·3 | 16·2 ± 5·5 | 145 ± 31 | 135 ± 37 |
| 72 | 13·4 ± 4·7 | 12·5 ± 3·4 | 116 ± 25 | 127 ± 30 |

tion as in short-term experiments. For cyclic AMP phosphodiesterase assay, skin squares were frozen between two plates of dry ice immediately after preincubation.

Hydrocortisone was dissolved in ethanol, the final concentration of each chemical being 100 μM and 0·5% (v/v) respectively. As control, only ethanol was added to the medium. The concentrations of adrenaline and histamine added for the cyclic AMP accumulation were 50 μM and 1 mM respectively. Previously it was shown that both these concentrations were sufficient for the maximal accumulation of cyclic AMP, which was reached after 5 min without phosphodiesterase inhibitors (Iizuka *et al.*, 1976; Yoshikawa *et al.*, 1975b).

The cyclic AMP content in these skin slices was measured by radio-immunoassay using a Yamasa cyclic AMP kit (Yamasa Shoyu Co. Tokyo, Japan) after partial purification by the method of Yoshikawa *et al.* (1975a, b). The assay system for the high and low km cyclic AMP phosphodiesterase has been described previously (Iizuka *et al.*, 1978b). Protein concentration was measured by the method of Lowry *et al.*, (1951). Chemicals and drugs were all prepared fresh before each experiment, and the pH of the medium was adjusted to 7.

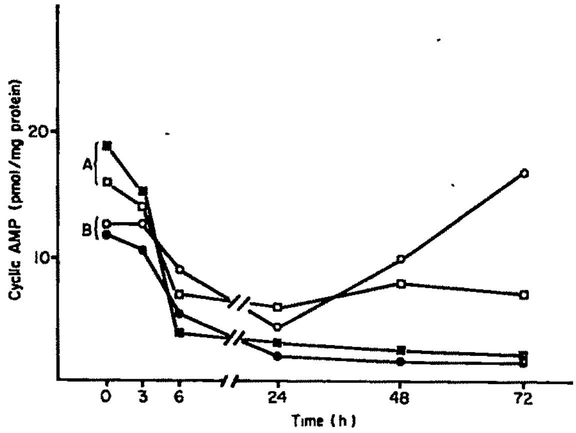

FIGURE 2. The effect of hydrocortisone on the adrenaline-adenylate cyclase system. Skin squares were incubated in Eagle's M.E.M. for an appropriate time with and without 100 μM hydrocortisone, and the adrenaline responses were examined. The concentration of adrenaline was 50 μM. Cyclic AMP level at 0 time means the value without incubation in Eagle's medium. Two independent experiments (A, B) are shown. In each series open circles or squares mean the values of hydrocortisone-treated skin, and closed circles or squares are values of controls.

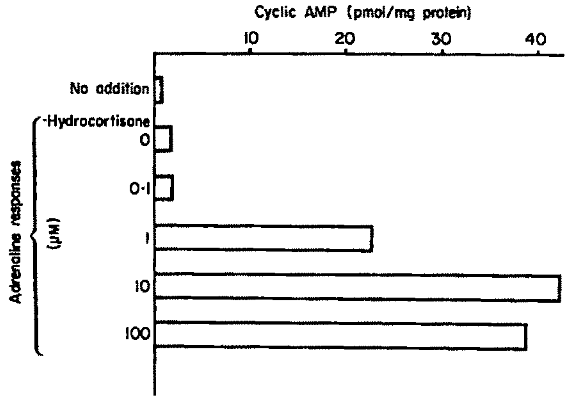

FIGURE 3. The concentration effect of hydrocortisone. Skin squares were incubated in Eagle's M.E.M. with different concentrations of hydrocortisone for 48 h. Skin squares were then incubated with 50 μM adrenaline for 5 min in the same way as in Fig. 2. Data are the means of three different experiments.

Adrenaline was the product of Daiichi Pharmaceutical Co. (Tokyo, Japan), hydrocortisone was from Sigma Chemical Co. (St Louis, Mo.), and Eagle's M.E.M. and Hank's medium were from Daigo (Osaka, Japan). All other chemicals were purchased from Wako Pure Indust. (Osaka, Japan).

## RESULTS

Figure 1 shows the effects of hydrocortisone on the accumulation of cyclic AMP in the skin. In these experiments, the skin squares were immediately pre-incubated in Hank's medium for 15 min, and then incubated for 5 min without phosphodiesterase inhibitors. As can be seen, neither ethanol nor hydrocortisone (with ethanol) had any effect by itself on cyclic AMP accumulation in the skin. Yoshikawa *et al.* (1976) reported that ethanol, at relatively higher concentrations, increased epidermal levels of cyclic AMP. At the low concentration (0·5% v/v) used in our experiments, ethanol had no effect on the cyclic AMP content of the skin.

Consistent with previous results (Yoshikawa *et al.*, 1975b; Iizuka *et al.*, 1976), both adrenaline and histamine markedly increased cyclic AMP levels in the skin. It was also shown that the addition of hydrocortisone to the medium did not alter the effects of ardenaline or histamine, and that the presence or absence of 100 $\mu$M hydrocortisone in the medium made no difference. With phosphodiesterase inhibitor (2 mM theophylline) in the medium, we also investigated the effects of hydrocortisone on adrenaline and histamine-induced cyclic AMP accumulation. Again the addition of hydrocortisone made no significant difference to the amount of cyclic AMP (data not shown). Thus it was shown that, in these short-term experiments, hydrocortisone had no effect on the accumulation of cyclic AMP in skin, when added to the medium either by itself or in combination with known stimulators of skin adenylate cyclase (adrenaline and histamine).

Consequently, we investigated the long-term effects of hydrocortisone (Table 1). Skin squares were incubated with and without 100 $\mu$M hydrocortisone in Eagle's M.E.M. and after 48 h they were incubated with either 50 $\mu$M adrenaline or 1 mM histamine (as noted in the Materials and Methods section). After this long-term incubation, the response to adenylate cyclase stimulators varied from experiment to experiment, especially in the case of histamine. This was probably due to the minor differences in the incubation conditions.

As can be seen, there was no significant difference between the basal levels of cyclic AMP in the skin with and without hydrocortisone treatment. Comparison of the responses to adrenaline shows that that in hydrocortisone-treated skin was 4·7 times greater than that in the control skin; this was statistically significant ($P<0{\cdot}02$). It was also noted that the response of control skin to adrenaline was much lower than that of the skin which had not been incubated long-term (Fig. 1; $P<0{\cdot}001$). On the contrary, responses of the hydrocortisone-treated skin to adrenaline were well preserved, and no significant difference was noted between the initial responses to adrenaline (Fig. 1) and those of hydrocortisone-treated skin after 48 h incubation. On the other hand the responses to histamine were well preserved during long-term incubation, and, although the mean value was slightly higher in the control skin, this was not statistically significant. We also investigated the effect of hydrocortisone on cyclic AMP levels in control skin after 48 h incubation. Again no increase in the level of cyclic AMP resulted from this hydrocortisone treatment.

Figure 2 shows the time course of the effect of hydrocortisone on the adrenaline-induced cyclic AMP accumulation. Two independent experiments (A and B) are shown. The incubations lasted 72 h, and the basal level of cyclic AMP was always around 1 pmol/mg protein. As can be seen there was a notable decrease in the response to adrenaline during the first 24 h both in control and in hydrocortisone-treated skin. However, this decrease was less marked in the hydrocortisone-treated skin. It was also less marked after 6 h incubation, after which time the response to adrenaline in the hydrocortisone-treated skin was always greater than in control skin. This tendency was most marked after 48–72 h, and in some experiments (as in Fig. 2B) the response to adrenaline in the hydrocortisone-treated skin was greater than the initial level of cyclic AMP.

Since the difference in response to adrenaline was marked after 48 h incubation, skin squares were incubated with various concentrations of hydrocortisone for 48 h, and with 50 $\mu$M adrenaline for 5 min. It was shown that the effect of hydrocortisone was almost concentration-dependent, and the maximal effect was observed with a concentration of 10 $\mu$M (Fig. 3).

The effect of hydrocortisone on cyclic AMP-phosphodiesterase is shown in Table 2. Both low and high Km enzyme activities decreased during the incubation period, and after 72 h incubation the enzyme activity was around 60% of the value without the long-term incubation. Although a slight decrease in high km enzyme activity was noted after 24 h in the presence of hydrocortisone, this was not statistically significant.

## DISCUSSION

Physiological concentrations of glucocorticoids are necessary before a number of metabolic effects mediated by other hormones can occur (Thompson & Lippman, 1974). This so-called 'permissive effect' has been seen in many cellular events including the cyclic AMP dependent process. Exton *et al.* (1972), for example, reported that glucocorticoids can correct the impaired stimulation of gluconeogenesis by either glucagon or adrenaline in livers of adrenalectomized animals. However, as the accumulation of cyclic AMP in liver (Exton *et al.*, 1972), heart (Miller, Exton & Park, 1971) or adipose tissue (Corbin & Park, 1969) was not inhibited in the adrenal deficient rats, they suggested that glucocorticoids act not on adenylate cyclase but on the metabolic process after cyclic AMP accumulates. Our data show that glucocorticoids affect the adrenaline-adenylate cyclase system in the skin.

Since levels of cyclic AMP were not elevated by the addition of hydrocortisone alone in either short-term (Fig. 1) or long-term experiments (Table 1), the effect of hydrocortisone is not due to the direct activation of adenylate cyclase in the skin. It was shown that the action of hydrocortisone on the adrenaline-adenylate cyclase system preserved adrenaline-induced accumulation of cyclic AMP (Table 1; Fig. 2).

Several investigators have reported the effects of glucocorticoids on the adrenaline (or $\beta$-adrenergic)–adenylate cyclase system. Manganiello & Vaughan (1972) reported that in cultured HTC hepatoma cells, after 72 h incubation with 10 $\mu$M dexamethasone, there was an increase in the amount of cyclic AMP produced by adding adrenaline plus theophylline. They also reported a decrease in phosphodiesterase activity with this addition of dexamethsone, and therefore suggested that the apparent increase in sensitivity to adrenaline (plus theophylline) was due to the glucocorticoid-induced decrease in phosphodiesterase activity. However, since theophylline is a strong inhibitor of phosphodiesterase, it is unlikely that reduced responsiveness to adrenaline plus theophylline is due to the altered activity of phosphodiesterase alone. Even if the dexamethasone decreases phosphodiesterase activity, much of it would be masked by the addition of theophylline.

On the other hand, Brostrom *et al.* (1975) reported that corticosterone increases basal and noradrenaline-stimulated levels of cyclic AMP in C6 glial-cell tumours. They also showed that there is no significant change in cyclic nucleotide phosphodiesterase activity. In our experiments, whose results are consistent with those of Brostrom *et al.* (1975), no significant difference was noted in the activity of phosphodiesterase. As the response to histamine was rather stronger in the control skin after long-term incubation (Table 1), it would seem that hydrocortisone acts on the adrenaline-adenylate cyclase system and not on cyclic nucleotide phosphodiesterase.

Contrary to the results of Brostrom *et al.* (1975), no significant difference was noted in basal levels of cyclic AMP (Fig. 1; Table 1). Moreover, the different responses to adrenaline, noted in long-term experiments (Table 1) were due mainly to the decreased response to adrenaline in the control skin. Glucocorticoid seems to act by protecting the adrenaline–adenylate cyclase system of the skin. On the other hand the histamine-adenylate cyclase system was much more stable whether or not hydrocortisone was present in the medium. Serum is known to contain many factors that are essential for the growth and survival of most mammalian cell lines (Gospodarowicz, Green & Moran, 1975). In our experiments, serum was not used because it might contain sufficient glucocorticoid to mask the effect of exogenous hydrocortisone. Flaxman & Harper (1975a) have reported on the culture of human skin in chemically defined media without serum. Under their conditions, which are very close to ours, they showed the epidermis to be well maintained for up to 7 days. There were no significant morphological changes for up to 72 h incubation in our experiments either. However, the lack of serum might affect the condition of the cells, and result in the decreased responsiveness to adrenaline. The phosphodiesterase activity also significantly decreased during the long-term incubation period (Table 2). Whatever may be the mode of action of glucocorticoids, our data show that they can, *in vitro*, regulate the adrenaline-adenylate cyclase system of the skin. Although it is usually suggested that glucocorticoids and the cyclic AMP system work independently, but in parallel (Voorhees *et al.*, 1975), this effect of hydrocortisone might be significantly involved in the skin's physiological regulatory system such as in cell proliferation and epidermal differentiation.

## ACKNOWLEDGMENTS

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*Case Reports*

# Steatocystoma multiplex with unusually prominent cysts on the face

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## SUMMARY

A patient is described who had unusually large facial cysts which histologically were typical of steatocystoma multiplex. An excellent cosmetic result was obtained after plastic surgery.

## CASE REPORT

M.P., a 42-year-old male from Jamaica, developed cysts on his face at the age of 22 years. Over the next 10 years these cysts enlarged considerably and he developed new lesions on the trunk. On occasions the cysts have discharged yellow oily material. There was no family history of similar lesions. Examination revealed numerous large cysts on the face; the largest of these being 2 cm in diameter (Fig. 1). Smaller cysts were scattered on the trunk and in the axillae. Histology of a lesion on the forehead revealed a thin-walled cyst in the mid dermis. The wall of the cyst contained abortive follicles and sebaceous glands (Fig. 2).

The lesions on the forehead were treated by plastic surgery with a most satisfactory cosmetic result. An incision was made in the hair-line and a flap of skin turned down; the cysts were then stripped from the dermal surface. There was incomplete clearance of some of the smaller cysts from the sides of the forehead. At follow-up 4 years later there was no obvious recurrence of the cysts in the surgically treated area (Fig. 3).

## COMMENT

In steatocystoma multiplex there are multiple dermal cysts which usually vary in size from a millet seed to a cherry stone (Bosellini, 1898). The wall of the cyst may contain any of the epidermal appendages, although the sebaceous gland is the most frequent. They are considered to be a form of hamartoma (Kligman & Kirschbaum, 1964). It is claimed that the condition is inherited as an autosomal dominant (Noojin & Reynolds, 1948; Anderson, 1950); however, as in our patient, one often fails to obtain a positive family history.

The distribution of the cysts tends to differ in males and females; in males they are found in the

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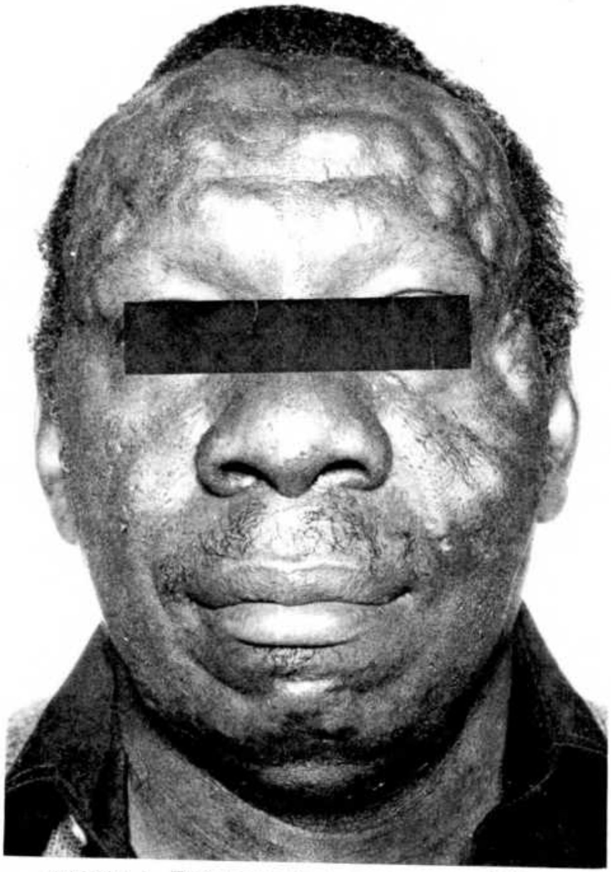

FIGURE 1. Facial cysts in steatocystoma multiplex.

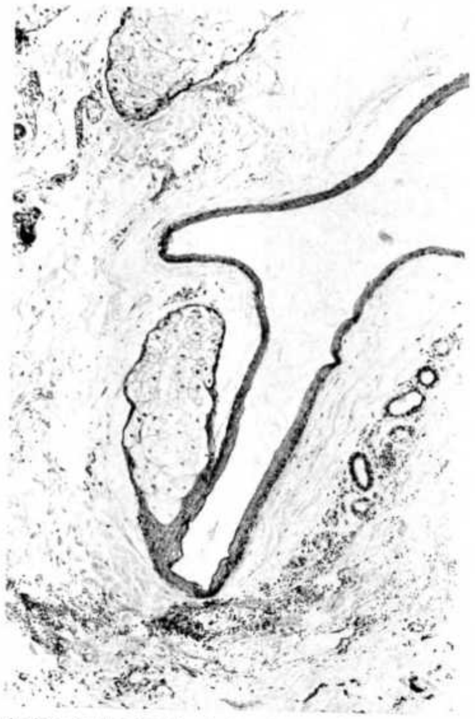

FIGURE 2. Histology of a lesion on the forehead showing a sebaceous gland adjoining the cyst wall.

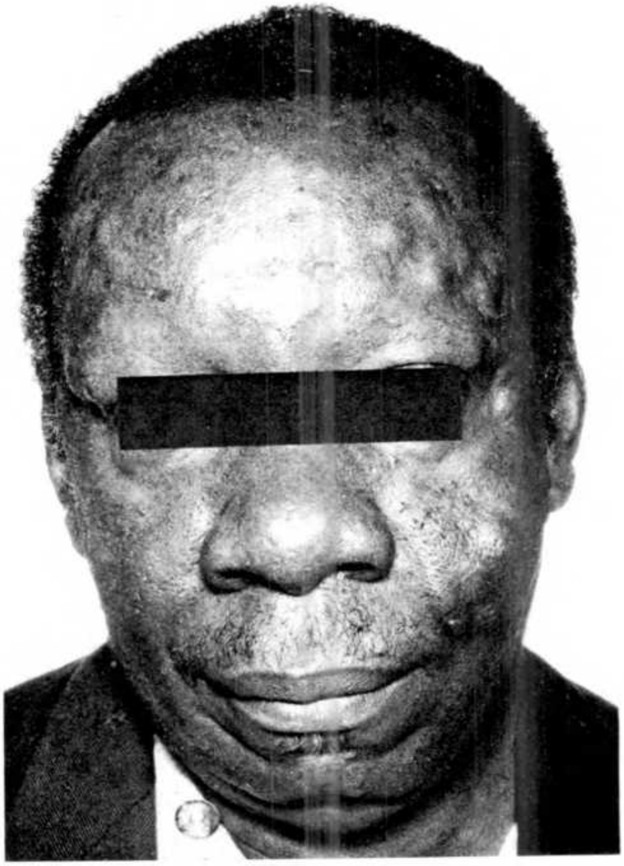

FIGURE 3. Facial appearance 4 years after surgery.

epigastrium and on the back, whereas in females they are more common in the axillae and groins. Cysts may be present on the face in both sexes; in a series of thirty-one patients there were eight patients with facial cysts (Leppard, personal communication). These cysts are usually clinically insignificant, but in our patient the most prominent cysts were on the face and these were unusually large.

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# Drug-induced anogenital cicatricial pemphigoid

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## SUMMARY

A case of cicatricial pemphigoid (CP) with a rare primary localization on the mucosal surfaces of the anus and the vulva and on perianal skin, associated with long-term therapy with clonidine, is described.

Direct immunofluorescence investigation of lesional skin suggests possible complement mediated tissue injury in between basement membrane and epidermal basal cells.

The possibility is discussed that drug induced metabolic disturbance (cyclic AMP-system) at a local level at the site of the basal cells might lead to the formation of immunogens and be one of the initiating factors in subepidermal bullous immune dermatoses.

It is concluded that in cases of cicatricial pemphigoid the use of drugs must be considered as one of the aetiological possibilities.

Cicatricial pemphigoid (CP) is a chronic disease of the mucosal surfaces and of skin, with a scarring nature, characterized by subepidermal blister formation (Lortat-Jacob, 1958; Lever, 1965; Hardy *et al.*, 1971). Using immunofluorescence (IF) techniques several authors have reported *in vivo* (direct IF technique) linear deposition of IgG and complement along the basement membrane zone of lesional skin (Bean, 1974; Cram, Griffith & Fukuyama, 1974; Griffith *et al.*, 1974; van Joost, Crone & Overdijk, 1976; Holubar & Stingl, 1976; Rogers *et al.*, 1977).

Rogers *et al.* (1977) provided data to suggest both classical and alternative pathway activation of complement in cases of CP.

From the literature it appears that the ocular and oral manifestations of CP are most common (Lever, 1965; Hardy *et al.*, 1971). The female genitalia as the site involved at the onset of CP has been described in four out of eighty-one cases examined by Hardy *et al.* (1971). CP of the anus and perianal tissue was reported in nine out of eighty-one cases examined by these authors.

In the present paper a case of CP with a rare site of onset is reported in a patient using the anti-hypertensive drug clonidine (Catapresan). Some aspects of the aetiology and mechanism are briefly discussed.

## CASE REPORT

A woman of 59 developed in January 1975 a painful eruption consisting of erythema, erosions and small blisters on the vulva. To a lesser extent some small erosions were present on the anal mucosa.

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Malignancy and a possible infectious aetiology could be excluded. Although the histopathology of a biopsy was not characteristic for kraurosis vulvae, this possibility was considered. For this reason and also because of severe cicatrization with shrinkage and chronic of burning pain, total vulvectomy was carried out (June 1975).

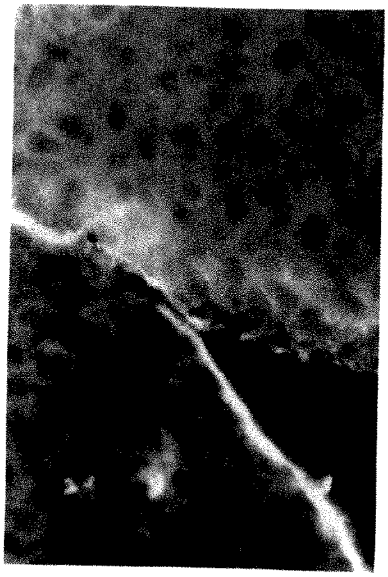

FIGURE 1. DIF test. Border of a subepidermal blister. Linear fluorescence (IgG) of the basement membrane zone at the bottom of the blister. IgG staining is also visible focally at the border of epidermal basal cells.

In December 1975 the patient was seen for the first time in our department and at that time there were small blisters with a positive Nikolsky phenomenon, several painful erosions and slight retraction of the anal mucosal surface and of perianal tissue, with spreading onto the perineum. Proctoscopy revealed a small number of erosions on deeper parts of the anal mucosa. No lesions of other mucosal orifices or of the skin were seen. At this time the patient had been treated with clonidine for hypertension for more than 2 years. No other drugs were being taken.

Light microscopic investigation of a biopsy of a perianal blister revealed subepidermal blister formation at the basement membrane zone. A cellular infiltrate consisting predominantly of lymphocytes and neutrophils and to a lesser extent of eosinophils was seen diffusely in the upper dermis and around blood vessels. In the biopsy examined no clear fibrosis of dermal tissue was seen.

By the direct IF test of perianal skin immediately adjacent to a bulla, linear deposition of immunoglobulins (IgG) and complement could be shown along the basement membrane zone. Other classes of immunoglobulins (IgM, IgA, IgD, IgE) were not demonstrable at this site (Cormane & Gianetti, 1971). These data established the diagnosis of CP. DIF tests with antisera to various components of complement (van Joost, Cormane & Pondman, 1972) revealed a linear deposition of C3 and C5 at the basement membrane zone (Fig. 1) with absence of subfactor C1q. At the site of the subepidermal blister the basement membrane fluorescence (IgG, C3 and C5) was observed only at the bottom of a blister. In this area and adjacent to the blister IgG and complement could also be shown at the outline of the basal cells (Fig. 1). Indirect IF tests were negative.

As a possible relation was considered between the appearance of lesions and the use of clonidine, the administration of this drug was stopped and bland local treatment was applied. Two months later no clear improvement of the anal and perianal lesions could be observed and the possibility of a drug induced reaction was considered very unlikely. Local treatment with betamethasone 17-valerate (Celestoderm) was started. Two months later anal and perianal lesions were healed apart from a slight focal retraction (van Joost *et al.*, 1977).

However, when about 1 year later the patient was re-exposed to oral clonidine therapy, sharply defined erythematous lesions appeared within a week, again especially in the anal and perianal region. This time, however, non-blistering erythematous lesions occurred also on the breast and abdomen. Because no other drugs were being used at that time the provisional clinical diagnosis was a drug reaction caused by clonidine. Further histological and immunological investigations were not possible at that time. The clonidine treatment was stopped and the lesions were treated locally with betamethasone 17-valerate and again all lesions, including anal and perianal lesions, disappeared within a week without scarring.

## DISCUSSION

In the present case the clinical and laboratory findings are in agreement with the diagnosis of anal, perianal and perhaps also vulval CP. It appears from the literature (Hardy *et al.*, 1971) that primary localization of CP on the mucous membranes of the vulva is rather rare and as far as we know CP with primary onset of lesions in anal and perianal regions has not been described before.

Reappearance of vivid erythematous lesions localized to the anal and perianal region after re-exposure to clonidine might be considered as highly suggestive of a relationship between the use of the drug and the earlier development of a syndrome with features of CP.

Clonidine is a well known $\alpha$-adrenergic receptor stimulating agent. Recently ocular CP has been described apparently due to long-term treatment with the $\beta$-adrenergic receptor blocking agent practolol (van Joost *et al.*, 1976). In this case psoriasiform skin lesions and serological features of lupus erythematosus (ANA and LE-cell phenomenon) were found as well.

The underlying mechanisms of side-effects on the skin of patients using $\beta$-adrenergic receptor blocking agents are not yet well understood, but it has been hypothesized that interaction with the cyclic AMP system of epidermal cells might be involved (Gaylorde & Sarkany, 1975).

A possible immunopathological relationship between bullous pemphigoid (BP) and CP has been discussed (Griffith *et al.*, 1974). Drug induced BP has also been described in the literature (Fellner & Katz, 1976). Glander & Barth (1975) reported a case of localized bullous fixed drug reaction with histopathological and immunological features of pemphigoid. Circulating basement membrane autoantibodies of the IgA class and high titre epidermal basal cell autoantibodies (IgG) have been reported in drug induced (phenylbutazone) skin lesions (van Joost, Asghar & Cormane, 1974). Serum auto-antibodies reactive with epidermal cytoplasmic components have been described in drug allergy (van Joost, 1974, 1979).

With respect to the localization of Ig and complement components at the site of both basement membrane and basal cells in our case (Fig. 1) the electron microscopic findings reported by Brauner & Jimbow (1972) are of interest. These authors reported in CP initial alterations of tonofibrils and hemidesmosomes of the basal cells and blister formation in between the lower membranes of the basal cells and the basement membrane. In the literature (Griffith *et al.*, 1974) a case of CP has been reported with circulating antibodies reactive with epidermal basal cells (indirect IF method).

Absence of factor C1q and the concomitant presence of IgG with complement components C3 and C5 at the basement membrane zone might be in favour of an alternative pathway activation of complement in this particular case of CP.

In view of the concept that at least a part of the basement membrane zone of skin is probably of epidermal origin, one may wonder if the underlying factors in CP and possibly also in BP might be related to local metabolic disturbances (cyclic AMP-system ?) of the germinal cells. This might lead to deposition of immunogens at the site of the basement membrane zone and subsequently to immune complex formation at this site.

In severe ocular or oral CP systemic therapy with azathioprine alone or in combination with corticosteroids has been recommended (Dave & Vickers, 1974). In the present case of apparently drug-induced CP, after stopping the administration of clonidine, local topical treatment with corticosteroids could stop the inflammatory process.

Generally in chronic blistering diseases with a tendency to scarring of the mucosal orifices, alone or in combination with skin lesions, IF investigations in addition to histopathological investigation must be performed to diagnose CP. The role of drugs as a triggering factor in the aetiology of subepidermal bullous immune dermatoses merits further study.

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# IgA bullous pemphigoid: a distinct blistering disorder

## CASE REPORT AND REVIEW OF THE LITERATURE

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### SUMMARY

We report a patient with an eccrine carcinoma who developed localized blistering which clinically resembled pemphigoid, histologically showed subepidermal blistering with features of both dermatitis herpetiformis and bullous pemphigoid, responded to dapsone and exhibited linear IgA deposition on direct immunofluorescence. The nosological position of patients with linear IgA deposition and subepidermal blistering is not clear. A review of the literature reveals that in adults linear IgA deposition may occur in three separate situations: dermatitis herpetiformis, bullous pemphigoid and a third condition of which our case is an example which is best termed IgA bullous pemphigoid. This condition is distinguished from cases of dermatitis herpetiformis with linear IgA by the clinical features and the site of IgA deposition on immunoelectronmicroscopy. It is distinguished from cases of bullous pemphigoid with linear IgA by the absence of circulating IgG antibasement membrane zone antibody, the therapeutic response to dapsone and the frequent occurrence of circulating IgA antibasement membrane zone antibody. IgA bullous pemphigoid has not previously been reported with a carcinoma but the association lends further support to the concept that this eruption represents a variant of pemphigoid.

It is customary to distinguish between dermatitis herpetiformis and bullous pemphigoid on the basis of four main criteria: clinical features, histological changes, immunofluorescence findings and therapeutic response. Additional criteria are the presence of jejunal changes in dermatitis herpetiformis and the association with HLA B8 antigen. Cases which exhibit features of both conditions have been termed intermediate or mixed cases. Initially this term was applied to conditions with the clinicopathological features of dermatitis herpetiformis which were shown by skin immunofluorescence techniques to be pemphigoid (Honeyman *et al.*, 1972). The term is now applied to cases which are not only clinically atypical, but show linear IgA deposition at the dermo–epidermal junction on direct

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skin immunofluorescence. In children this particular entity has been termed chronic benign bullous dermatosis of childhood (Faber & Van Joost, 1973; Prystowsky & Gilliam, 1976; Clayton, 1976; Esterly *et al.*, 1977). IgA bullous pemphigoid (Provost *et al.*, 1979) would seem the most appropriate title for the adult cases.

## CASE REPORT

A 76-year-old female patient of Polish extraction presented with an 8-year history of a slowly enlarging solitary lesion on the left side of the abdomen. A year previously the lesion had ulcerated, since which time the patient had been applying bismuth powder. In 1952 a left acoustic neuroma had been removed surgically leaving her with a left facial palsy.

Examination revealed an ulcerated nodular lesion of 4 cm diameter on the left side of the abdomen. Within a week of presentation the patient developed pruritus and blistering which were located mainly around the ulcerated lesion. The eruption was monomorphic and consisted of clear fluid filled blisters approximately 1 cm in diameter which appeared on a slightly erythematous background. A few bullae were also present over the right clavicle but the face and limbs were spared and there was no mucous membrane involvement.

### *Investigations*

*Biopsy of the ulcerated nodule*. Histology showed islands of abnormal cells occupying the full thickness of the dermis (Fig. 1) with lymphatic involvement along the deep aspect of the tumour. The epidermis overlying the central tumour mass was eroded. Adjacent areas showed collections of atypical cells within the epidermis (Fig. 1). Primitive ductal structures were visible within the islands of abnormal cells which contained considerable numbers of large cells with clear cytoplasm, some of which were PAS positive. The lesion was reported as a poorly differentiated malignant sweat gland tumour.

*Biopsy of blister*. Histology showed a large subepidermal bulla containing predominantly eosinophils, and a sparse upper dermal infiltrate consisting of mononuclear cells and eosinophils. Collections of cells were seen at the tips of the dermal papillae adjacent to the main bulla (Fig. 2). These collections consisted mainly of eosinophils with only an occasional neutrophil.

*Immunofluorescence studies*. Direct immunofluorescence of perilesional skin showed linear deposition of IgA and C3 at the dermo–epidermal junction on two separate occasions.

Indirect immunofluorescence using normal human skin as a substrate was negative on two separate occasions for IgA, IgG and C3. Immunofluorescence studies on tumour tissue were negative.

*Further investigations*. Haemoglobin: 13·0 g/100 ml. White count: 7400/mm$^3$. ESR: 59 mm/h. Serum immunoglobulins: normal. There was no clinical evidence of malabsorption. Endoscopy and jejunal biopsy were normal. HLA tissue typing revealed A1 and B8 antigen.

*Progress and management*. Therapy with dapsone 50 mg twice daily produced prompt relief from itching and no further blisters developed. Wide excision of the tumour was performed. Subsequent withdrawal of dapsone was followed within 10 days by further blistering at the same sites as before.

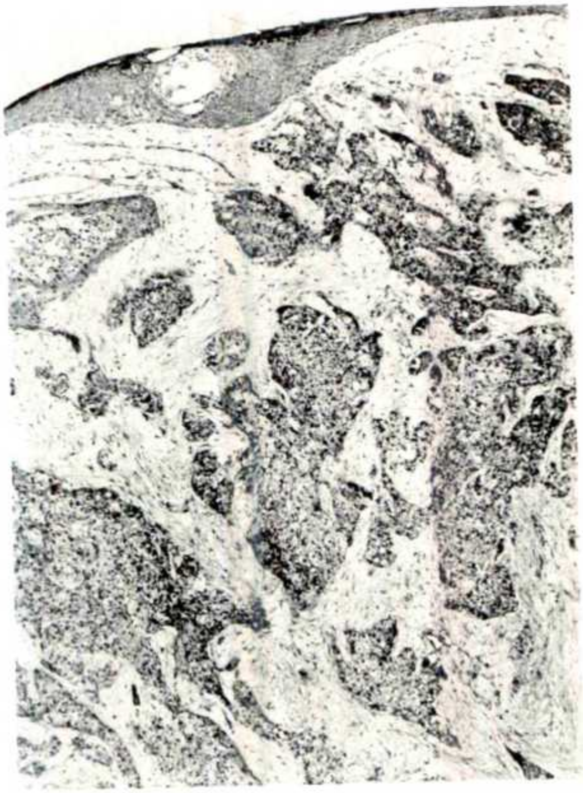

FIGURE 1. Section of eccrine carcinoma. Medium power view to show islands of malignant cells occupying the dermis with large numbers of clear cells. There is a nest of atypical cells within the epidermis (× 38).

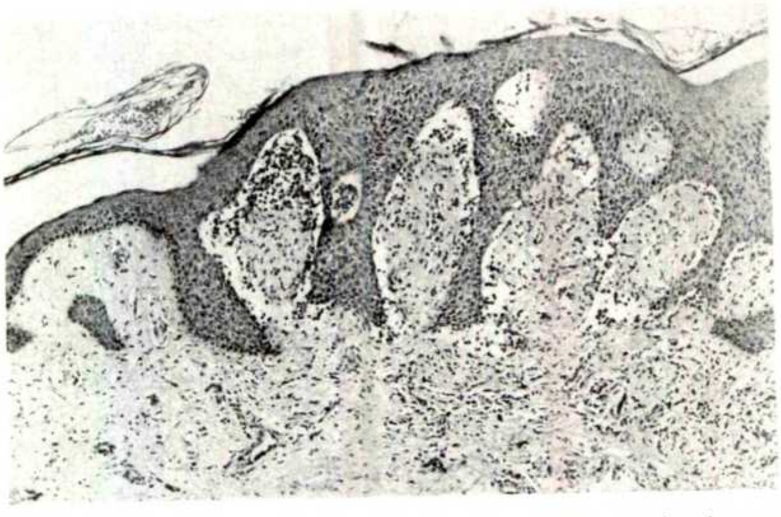

FIGURE 2. Section of skin adjacent to blister. View to show papillary tip microabscesses (× 65).

## DISCUSSION

The age of onset, distribution and morphology of our patient's eruption were more in keeping with bullous pemphigoid, whereas the pruritus, dapsone responsiveness and HLA type were more in favour of dermatitis herpetiformis. Histologically the papillary tip microabscesses were suggestive of dermatitis herpetiformis, whereas the predominance of eosinophils and the large subepidermal blister were more in keeping with bullous pemphigoid. Although it might be argued that the immunofluorescence findings merely highlighted this dilemma, the presence of linear IgA in fact established the diagnosis of IgA bullous pemphigoid.

In adults there are three situations in which linear IgA has been recorded. Firstly a proportion of patients with clinically and histologically typical dermatitis herpetiformis have linear rather than granular IgA deposition (Seah *et al.*, 1972; Seah & Fry, 1975) and the proportion of such cases may be as high as 18% (Katz & Strober, 1978). Immunoelectron microscopic studies demonstrate that the IgA deposition is below the basement membrane (Yaoita & Katz, 1976) as in ordinary dermatitis herpetiformis (Stingl *et al.*, 1976). The second situation in which linear IgA may be found in adults is in association with bullous pemphigoid. Approximately 20% of patients with clinically and histologically typical pemphigoid have a band of linear IgA on direct immunofluorescence (Ahmed, Maize & Provost, 1977). These cases are readily distinguishable from patients with dermatitis herpetiformis as linear IgG is present as well. Indirect immunofluorescence may also be of value as circulating IgG antibasement membrane zone antibody is present in 80% of cases of bullous pemphigoid. Furthermore, these cases are steroid responsive and do not exhibit a gluten sensitive enteropathy. Electronmicroscopic studies reveal that the site of immunoglobulin deposition and the level of dermo-epidermal separation is located in the region of the lamina lucida (Kobayasi, 1967; Braun-Falco & Rupec, 1967; Schaumberg-Lever *et al.*, 1975; Schmitt-Ullrich *et al.*, 1975; Holubar *et al.*, 1975).

No suitable title exists for the third situation in which linear IgA deposition occurs in adults, so previous authors have attempted to designate all such cases as examples of either dermatitis herpetiformis or bullous pemphigoid. This has probably tended to confuse rather than clarify the situation particularly in relation to jejunal pathology and HLA typing. Such cases have been reported as intermediate or mixed forms (van der Meer, 1969; Jablonska *et al.*, 1976), atypical or linear dermatitis herpetiformis (Yaoita, Hertz & Katz, 1976), polymorphic pemphigoid (Honeyman *et al.*, 1979) and IgA bullous pemphigoid (Provost *et al.*, 1979). Early reports are difficult to evaluate as it was not until 1976 that immunoelectron microscopic studies demonstrated that linear IgA deposition could occur on two different levels (Yaoita & Katz, 1976). Electron microscopic studies by other workers showed that cases with clinically typical dermatitis herpetiformis have a dermal pathology whereas the atypical cases show changes similar to pemphigoid (Dabrowski *et al.*, 1977). Jejunal changes were confined to the former group and immunoelectronmicroscopic studies of an atypical case demonstrated linear IgA deposition within the lamina lucida (Dabroski *et al.*, 1978). The probability that clinically atypical cases with linear IgA are in fact variants of pemphigoid has been confirmed more recently. Provost *et al.* (1979) reported two cases of IgA bullous pemphigoid with clinical features remarkably similar to our patient, one of whom had circulating IgA anti basement membrane zone antibody, and in the other immunoelectron microscopic studies showed localization of the IgA within the lamina lucida. Similarly Honeyman *et al.* (1979) described six cases of polymorphic pemphigoid with linear IgA deposition of whom four were dapsone responsive and so fulfil our criteria for the diagnosis of IgA bullous pemphigoid. Circulating IgA antibasement membrane zone antibody was present in two of these cases.

On this basis the patient with linear IgA lamina lucida deposition reported by Yaoita *et al.* (1976) we would classify as having IgA bullous pemphigoid. Unfortunately the situation is confused by the finding of circulating IgA antibasement membrane zone antibody in the sera of three patients considered to have dermatitis herpetiformis (Yaoita & Katz, 1977) each of whom had a different pattern of IgA deposition: granular, linear sub-basal and lamina lucida. The authors accept that these findings are difficult to interpret and in a later review concede that their cases of dermatitis herpetiformis may result from more than one patho-physiological process (Katz & Strober, 1978).

There may be some clinical overlap between IgA bullous pemphigoid and atypical cases of IgG bullous pemphigoid (Honeyman *et al.*, 1979). However, we would maintain that the distinction is still valid because only a small percentage of patients with IgG pemphigoid respond to dapsone (Person & Rogers, 1977). Furthermore circulating IgA antibasement membrane zone antibody is a frequent finding in IgA bullous pemphigoid though it is not present in all cases and was not present in our case. The relationship of IgA bullous pemphigoid with gluten sensitive enteropathy has not finally been established but no jejunal pathology has yet been found in patients with linear IgA in whom lamina lucida deposition has actually been confirmed by immunoelectron-microscopy. Our patient carries B8 antigen but there was no evidence of gluten sensitive enteropathy on endoscopy or jejunal biopsy.

There are obvious similarities between this adult disorder and the condition of chronic bullous dermatosis of childhood which has itself been termed juvenile dermatitis herpetiformis (Grant, 1968) and juvenile pemphigoid (Faber & van Joost, 1973). Both conditions are clinically atypical dapsone responsive bullous dermatoses with histological features of both dermatitis herpetiformis and bullous pemphigoid (Bean & Jordon, 1974). Linear IgA deposition is characteristic (Marsden, Skeete & Black, 1979), circulating IgA antibasement membrane zone antibody has been recorded (van der Meer *et al.*, 1977) and ultrastructural studies have shown separation within the lamina lucida (Ortonne, Schmitt & Jacqueline, 1978). Furthermore immunoelectron microscopic studies of one childhood case has demonstrated that IgA deposition occurs within the lamina lucida as in adult cases of IgA bullous pemphigoid (Dabrowski *et al.*, 1979). It would therefore seem reasonable to classify chronic bullous dermatosis of childhood as a juvenile form of adult IgA bullous pemphigoid.

Finally, one has to consider the curious association of our patient's eruption to the malignant sweat gland tumour. This lesion was difficult to classify precisely. The nests of atypical cells seen within the epidermis were reminiscent of an eccrine poroma, but this type of Borst-Jadassohn effect may also be seen with epidermotrophic tumours arising within the dermis (Mehregan & Pinkus, 1964). In our patient the epidermal erosion was too extensive for these two possibilities to be differentiated. It is difficult to dismiss the association of the tumour with the bullous eruption as fortuitous since our patient's blisters were related geographically to the site of epidermal ulceration. Immunologically there seemed to be no cross-reaction between the tumour cells and normal skin as direct immunofluorescence of the tumour was negative. Another possibility is that the epidermotrophic tumour might have exposed hidden basement membrane antigen and thus evoked in turn the production and deposition of antibasement membrane zone antibody. Finally one could regard this phenomenon as yet another example of the putative association of pemphigoid with an underlying malignancy. At present, however, such mechanisms remain speculative.

## ACKNOWLEDGMENTS

We would like to thank Dr David Lovell for his invaluable assistance with the immunofluorescent and histological studies.

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**Society Proceedings**

# British Association of Dermatologists Investigative Group — Winter Meeting, Glasgow, 18–19 January 1980

## SUMMARIES OF PAPERS

**Friday 18 January**

**SESSION 1** *Chairman* PROF. M.W.GREAVES

### (1) The relapse of plaque psoriasis after clearing with photochemotherapy or dithranol

D. VELLA BRIFFA*, M.W.GREAVES*, A.P.WARIN*, SARAH ROGERS†, JANET MARKS† AND S.SHUSTER†
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The aim of this investigation was to examine the ability of photochemotherapy to induce a remission of severe plaque psoriasis without using maintenance treatment, and to compare it with that of the 'Ingram' dithranol regimen. Fifty-three patients with plaque psoriasis affecting on average 30% of the body area were cleared with photochemotherapy using oral 8-MOP (0·6 mg/kg body weight) and UV-A from PUVA '4000' and '6000' units (H. Waldmann, Germany). Treatment was subsequently stopped, and recurrence of the rash was assessed at monthly intervals as a percentage of the body area affected before the start of treatment. Patients were followed for a range of 8–16 months, and during this period psoriasis relapsed to 50% of its pre-treatment area in thirty-seven patients (70%) in a mean of 6·3 months (s.e. mean 0·4). Of these, twenty patients were treated with photochemotherapy again and they all cleared. These results were compared with the outcome in seventy-six patients with psoriasis of equal severity who were followed for a range of 4–16 months after clearing with dithranol. Psoriasis relapsed to 50% of its pre-treatment area in fifty-six patients (74%) in a mean of 5·4 months (s.e.mean 0·4). The remission time after a course of photochemotherapy or dithranol is comparable. It now remains to be shown whether intermittent courses of photochemotherapy or continued maintenance treatment is the better way of using this treatment in plaque psoriasis.

### (2) Photochemotherapy of Psoriasis: DNA damage in blood lymphocytes

P.S. FRIEDMANN AND SARAH ROGERS
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The effects of photochemotherapy (PUVA) with 8-methoxypsoralen (8-MOP) and UV-A were observed upon phytohaemagglutinin (PHA)-induced DNA synthesis by lymphocytes from patients with psoriasis. The emission from the UV-A source contained sufficient short wavelengths (UV-B) to impair $^3$H-thymidine incorporation by lymphocytes irradiated *in vitro* in the absence of 8-MOP. The effect of these wavelengths could be prevented by irradiating cells through clear plastic screens.

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When lymphocytes from patients receiving PUVA treatment were irradiated *in vitro* in a 1/10 dilution of plasma containing 8-MOP, increasing doses of UV-A produced progressive inhibition of PHA-induced DNA synthesis. Cells from patients given several previous treatments were inhibited significantly more than those from patients receiving their first exposure ($P < 0{\cdot}01$ by analysis of variance).

To examine the effects of 8-MOP and UV-A *in vivo*, lymphocytes were collected from patients receiving PUVA therapy just before and again immediately after patients were irradiated. Cells taken after irradiation gave lower levels of both spontaneous and PHA-induced $^3$H-thymidine incorporation than those taken before irradiation. This was found in patients receiving their first exposure as well as those who had received several previous treatments. Cells taken prior to irradiation from patients who had received several treatments gave significantly lower $^3$H-thymidine incorporation (geometric mean 8425 c.p.m., range of s.e.mean—7406–9585) than those from patients about to receive their first treatment (geometric mean 15, 108 c.p.m., range of s.e.mean—13, 662–16, 709) ($P < 0.005$ by Student's '*t*'-test). Thus repeated exposure to PUVA *in vivo* resulted in impaired lymphocyte responses to PHA.

## (3). Drug oxidation in psoriasis

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We have previously shown that the activity of aryl hydrocarbon hydroxylase is reduced in the epidermis of psoriatic both lesions and in the clinically normal, lesion free skin of psoriatics when compared to normal epidermis. In order to examine whether or not this reflects a widespread impairment of metabolism, we have studied the pharmacokinetics of debrisoquine and antipyrine oxidation in patients with psoriasis when compared to non-psoriatic individuals.

Debrisoquine 4-hydroxylation is under the control of a single gene locus with 9% of the British population homozygous for poor metabolism. Untreated patients with severe discoid psoriasis were given 10 mg debrisoquine orally after an overnight fast. Urine was collected over the subsequent 8 h, and analysed for debrisoquine and 4-hydroxydebrisoquine by gas-liquid chromatography. The metabolic ratio was calculated from the quotient of the % dose eliminated as debrisoquine and the % dose eliminated as 4-hydroxydebrisoquine. In psoriatics the incidence of phenotypically poor metabolisers (metabolic ratio $> 12{\cdot}5$) and the frequency distribution of the extensive metabolizers did not differ from that of 258 healthy controls.

Antipyrine kinetics were studied in a further group of untreated patients with severe discoid psoriasis. Patients were given 600 mg of antitipyrine orally after an overnight fast and six saliva samples collected over the subsequent 32 h and analysed for antipyrine by gas liquid chromatography. There was no significant difference in the clearance of antipyrine between psoriatics and normal controls.

We therefore conclude that as the *in vivo* oxidation of debrisoquine and antipyrine are not impaired in patients with severe discoid psoriasis, there does not appear to be a widespread defect in oxidation in this disorder.

## (4) Leukocyte migration and phagocytic function in psoriasis

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Transepidermal neutrophil leukocyte migration is a characteristic feature of psoriasis. Psoriatic scale has been shown to contain leukotactic factors; however, reports on intrinsic neutrophil chemotaxis and phagocytic activity *in vitro* in psoriasis have been conflicting. We have therefore studied neutrophil function in psoriatic subjects both *in vitro* and in uninvolved skin *in vivo*.

*In vivo* migration of neutrophils was assessed by the Segal and Loewi quantitative skin window chamber technique on uninvolved forearm skin of thirty psoriatic subjects and thirty healthy volunteers. Mean random cell migration towards buffer alone in the chamber, and mean chemotactic migration towards 50% autologous serum in the chamber, were not significantly different between the two groups.

*In vitro* random migration and chemotaxis towards endotoxin-activated serum and casein were measured by a modification of Wilkinson's method, using the leading front technique, in thirty psoriatic and forty control subjects, and were not significantly greater in the psoriatic group. No effect of psoriatic serum on control cell chemotaxis was demonstrated.

*In vitro* phagocytosis of *Candida albicans* was studied in thirty psoriatic and thirty control subjects, and was not found to be significantly enhanced in the psoriatic group or when control cells were preincubated in psoriatic serum.

These results contradict reports of abnormal leukocyte chemotaxis and phagocytosis in psoriasis and support the theory that leukocyte migration into psoriatic lesions *in vivo* is the result of local accumulation of leukotactic factors rather than of intrinsic enhancement of chemotactic activity.

### (5) The assessment of metaphase degeneration in psoriatic epidermis during measurement of the birth rate with vincristine

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The accurate measurement of epidermal proliferative rates in psoriasis is very difficult, but the metaphase arrest method with intradermal vincristine has emerged as a reliable method for measuring the *birth rate* (Duffill *et al.*, 1977, *British Journal of Dermatology*, **96,** 493). However, errors are possible if arrested metaphases degenerate and become unrecognizable during the experimental period.

We have measured the life-span and rate of metaphase generation in patients with stable plaque psoriasis. Multiple sites on the periphery of the plaques were injected at the same time with 1 $\mu$Ci of tritiated thymidine and 1 $\mu$g of vincristine sulphate; biopsies were taken at regular intervals for times up to 10 h after injection and processed for autoradiography. Separate counts of labelled, unlabelled and total arrested mitoses were made.

The cohort cells in the G2 phase at the time of injection appear as arrested, but unlabelled mitoses after vincristine; thus the fate of this cohort can be followed. Initially G2 cells flowed linearly into mitosis, but after 4 h the number of unlabelled mitoses began to decrease, indicating that vincristine-arrested metaphases had a minimum life span of about 4 h, and disappear at a rate of 0·43% per hour.

We conclude that metaphase-arrested experiments in psoriatic epidermis should be confined in length to 4 h; however, if longer periods are required, then correction factors are readily calculable. However, if experiments are extended beyond 4 h, and correction factors not used, then serious errors are incurred (Fisher, *British Journal of Dermatology*, **80,** 235).

## SESSION 2

*Chairman* DR N. PLUMMER

### (6) The effect of hyperplasia upon epidermal keratinization

IRENE HUNTER AND D.SKERROW
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The polypeptide chain composition of human epidermis has been examined after repeated removal of the stratum corneum by scraping or sellotape stripping. This was to determine whether the degree of hyperplasia induced by these procedures has a detectable effect upon keratinization and, if so, whether this is comparable with that seen in hyperplastic skin conditions.

The stratum corneum was removed at 48 h intervals, extracted, and examined by sodium dodecyl sulphate gel electrophoresis. Initially the extracts contained the two major polypeptide chains, molecular weights 70K and 60K, found in normal stratum corneum. With repeated stripping, the amount of the

60K chain progressively decreased and that of two other chains, molecular weights 63K and 55K, normally found only in epidermal prekeratin, increased. These results illustrate that, during normal keratinization, two of the three-chains of prekeratin are modified: the 55K chain is removed or cross-linked so that is it not present, as such, in the stratum corneum and the 63K chain is modified so as to have an apparent molecular weight of 60 K. During induced hyperplasia, these changes are incomplete and relatively unmodified prekeratin molecules persist into the stratum corneum.

The polypeptide chain composition of the stratum corneum produced after stripping is clearly different from that produced in chronic plaque psoriasis but is similar to that seen in several other conditions believed to involve hyperplasia. It appears therefore that the molecular abnormality observed in psoriasis cannot be produced as a consequence of hyperplasia alone, but involves an additional defect of keratinization.

## (7) Cytoplasmic glycoproteins in the germinative compartment of the human epidermis: tracing with monoclonal antibodies

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The cold agglutinins (C.A.) produced during chronic cold agglutinins disease (C.C.A.D.) are monoclonal immunoglobulins. These C.A. were amongst the first monoclonal immunoglobulins with a known antibody specificity; they react with several (at least three) systems of glycoprotein antigens (Pr; Ii; Gd) situated on the surface of red blood cells. Antigens reacting with C.A. of C.C.A.D. have also been detected in the kidney. The expression and localisation of these antigens in the human skin have not been demonstrated previously.

We have studied fourteen sera from patients with C.C.A.D. using indirect immunofluorescence. (These sera were kindly provided by Dr Habibi, CNTS, Paris.) Monoclonal C.A. in these sera reacted either with Pr antigens or with I and/or i antigens, as shown by extensive agglutination tests using enzyme treated red blood cells; the haemagglutination titres of anti Pr, anti I and anti i sera were similar (1/16000).

Only anti Pr sera were found by I.I.F. to react with normal human skin specimens. The immunofluorescent staining was observed only with the anti-light or anti-heavy chain labelled antisera corresponding to the monoclonal immunoglobulin with anti Pr activity. The staining was abolished by previous treatment of the substrate with papain, trypsin or neuraminidase. Finally, eluates (from Pr specific red blood cell agglutinations) were found to give similar immunofluorescent staining. All these controls demonstrated the Pr specificity of the staining.

Most importantly the Pr antigens were localized in the basal layer of normal human skin (B.C.L.); they were not detectable in the prickle cell layer in the granular layer, or in the horny layer. The staining pattern in the B.C.L. was consistent with a cytoplasmic localization of the Pr antigens.

The antigenic determinants of the Pr antigens consist of N-acetyl neuraminic acid bound $\alpha$ ketosidically to the oligosaccharide chains of glycoproteins. Our data show that the glycoproteins of the B.C.L. may differ from those of the other layers of the epidermis in terms of antigenic properties. This is also the first immunochemical evidence that the 'basal cytoplasmic pattern' observed in I.I.F. with sera containing the so-called 'B.C.L.' antibodies may reflect biochemical characteristics of the germinative layer which are not detected by other techniques.

## (8) The suppression of sebaceous gland activity by $H_2$ receptor antagonists

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We have reported that the $H_2$ receptor antagonist cimetidine suppresses sebaceous gland activity in patients with acne. To see whether this property is shared by other drugs of this class we have studied the skin before and during treatment with ranitidine in twelve patients with acne for 3 months in a dose of 150 mg twice a day for the first and third months and 120 mg twice a day during the second month. For comparison twelve similar patients were given cimetidine 1 g daily for the same period; antibiotic dosage was unaltered. An early and sustained reduction in seborrhoea was noted by most patients in both groups in contrast to a slight and more delayed improvement in acne in about half. The mean reduction in sebum excretion was 25% for ranitidine and 35% for cimetidine and both were significantly different from pre-treatment rates ($P < 0{\cdot}01$) and from each other ($P < 0{\cdot}05$). The three-fold greater molar potency of the two blockers in the doses studied is less than that for $H_2$ receptor blockade in other tissues. Pharmacokinetic factors could account for this but an alternative possibility is that sebaceous gland inhibition is not due to $H_2$ receptor blockade but to an unrelated consequence of the chemical similarity of both agents. To resolve this, further studies of $H_2$ antagonists of more widely dissimilar structure and potency are now required.

## (9) The preputial glands and the cyclical release of sex attractants by the female rat

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Odours released by female rats play an important role in attracting sexually experienced males. In this study we have investigated the relationship between the release of such odours and the oestrus cycle and the possibility that the preputial glands are a source of these odours.

The test system used has been described previously (Thody & Dijkstra, 1978, *Journal of Endocrinology*, 77, 397) and involves the presentation of two female rat odour sources to ten successive pairs of sexually experienced males and the measurement of investigating times.

Whole body odours of female rats at different stages of the oestrus cycle were compared with those of ovariectomized controls. Male rats preferred the odours of mid and late pro-oestrus females ($P < 0{\cdot}01$ in both cases) while those of metroestrus and dioestrus females were relatively unattractive. Thus in the female rat sex odours may show a cyclical pattern of release.

The possible involvement of the preputial glands was investigated by comparing the whole body odours of females in late pro-oestrus and late metoestrus before and after preputialectomy. Male rats preferred the odours of pro-oestrus females to those in metoestrus ($P < 0{\cdot}01$) but after preputialectomy odour attractiveness was reduced in both groups ($P < 0{\cdot}01$ in both cases) and the preference for pro-oestrus females was no longer observed.

We conclude that the preputial glands are a major source of sex attractants in the female rat and may be responsible for the changes in female attractiveness that occur during the oestrus cycle.

## (10) The effect of melanotrophin potentiating factor (MPF) on the pigmentary action of neuroleptics on *Anolis* skin

R.J.Carter and S.Shuster
*University Department of Dermatology, Newcastle-upon-Tyne*

MPF has been defined as the C-terminal tetrapeptide of human $\mu$-lipotrophin and it potentiates the action of melanocyte-stimulating hormones (MSHs). Neuroleptics mimic MSH action on *Anolis* skin and, in the present study, we have demonstrated that MPF also potentiates neuroleptic pigmentary action.

Dose–response curves were obtained for $\alpha$-MSH, chlorpromazine, perphenazine, prochlorperazine, trifluoperazine and haloperidol in the presence and absence of MPF using the *in vitro Anolis* rate bioassay. Using analysis of variance, neuroleptic potencies were calculated relative to $\alpha$-MSH. Potencies of each pigmentary agent with MPF were calculated relative to the pigmentary agent on its own.

Neuroleptic and $\alpha$-MSH dose-response curves were parallel and the relative potencies were $\alpha$-MSH, 1·00; trifluoperazine, 13·1 × $10^{-6}$; perphenazine, 2·73 × $10^{-6}$; haloperidol, 1·61 × $10^{-6}$; chlorpromazine, 1·49 × $10^{-6}$ and prochlorperazine, 1·08 × $10^{-6}$. Both 250 × $10^{-12}$ M and 500 × ${}^{10-12}$ M concentrations of MPF caused significant ($P < 0{\cdot}01$) dose-related shifts of the $\alpha$-MSH and neuroleptic dose-response curves in parallel to the left. Thus MPF potentiated neuroleptic action and the relative activity of MPF on each pigmentary agent was trifluoperazine, 1·00; perphenazine, 0.906; $\alpha$-MSH, 0·833; chlorpromazine 0·676; haloperidol, 0·666 and prochlorperazine, 0·545.

The present results confirm direct pigmentary action of neuroleptics and, since their potencies correspond with their therapeutic potencies in man, *Anolis* skin darkening may be a useful drug secreening method. The results also suggest possible brain interactions between MSH, MPF and the neuroleptic drugs.

## SESSION 3

*Chairman* PROF. S.SHUSTER

### (11) Ultrasound detection of corticosteroid induced dermal atrophy

C.Y.TAN, P.DYKES AND R.MARKS
*Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff*

The xeroradiographic technique (Marks, Dykes & Roberts 1975, *Archives for Dermatological Research*, **253**, 93) and a pulsed ultrasound technique (Alexander & Miller, 1979, *Journal of Investigative Dermatology*, **72**, 17) have been used in forty-eight normal individuals in order to assess the usefulness of the ultrasound technique and monitor corticosteroid induced skin atrophy from the topical application of six preparations (1% hydrocortisone, 0·1% hydrocortisone 17 butyrate, 0·05% clobetasol propionate, 0·05% clobetasone butyrate, 0·1% betamethasone valerate and bland unmedicated cream). The study was conducted according to a randomized double blind design with each subject using two of the preparations, one on either forearm. Xeroradiographic measurements were made before starting and after 6 weeks of treatment. The two techniques gave similar results when the pre- and post-treatment measurements were correlated (pre-treatment $r = 0{\cdot}89$; post-treatment $r = 0{\cdot}96$). Ultrasound measurements detected significant decreases in skin thickness at all times after 2 days in sixteen subjects who applied clobetasol propionate. It was concluded that the pulsed ultrasound technique was a reliable, accurate non-invasive technique for the measurement of dermal thickness which does not employ ionizing radiation and which is capable of yielding information of considerable importance on the kinetics of alteration of dermal connective tissue mass.

### (12) Plasma zinc and whole blood delta-aminolaevulinic acid dehydratase levels in psoriasis and mycosis fungoides

MARTINE A.HUGHES*, M.E.IMOGEN MORGAN*, E.M.MCMILLAN†, HELEN M.NELSON† AND RONA M. MACKIE†
**University Department of Materia Medica, Stobhill General Hospital, Glasgow, and* †*University Department of Dermatology, Western Infirmary, Glasgow*

Zinc compounds are major components of many topical skin preparations. The effects of oral zinc supplementation in wound healing and in skin diseases have beeen well documented, but the possibility that the local application of zinc compounds to diseased skin might have a therapeutic effect through percutaneous absorption has not been widely explored.

Plasma zinc, plasma copper and blood delta-aminolaevulinic acid dehydratase (delta-ALAD, a haem enzyme which is known to be zinc dependent in physiological concentrations in man) were investigated in thirty psoriatic in-patients during treatment with compounds containing zinc oxide. No significant alterations in these parameters occurred over the 2 week study period.

Significantly reduced plasma zinc levels were found in the patients even after clinical improvement, compared to a matched control population. Other psoriatic patients receiving photochemotherapy (PUVA) only also had significantly reduced levels of plasma zinc. Blood delta-ALAD levels in topically treated patients were within the normal range but those of the patients receiving photochemotherapy were significantly elevated.

By comparison, groups of patients with mycosis fungoides on photochemotherapy and on topical therapy were studied; delta-ALAD levels were reduced in both these groups.

The clinical relevance of these abnormalities of zinc distribution and of delta-ALAD disturbance in skin diseases remains to be elucidated.

### (13) Production of antibodies on the bovine skin in response to vaccination

D.H.LLOYD AND D.MCEWAN JENKINSON
*Department of Physiology, The Hannah Research Institute, Ayr*

The immunoglobulins (Ig)A, G and M on cattle skin and the apparent local production of IgA indicate the presence of specific surface humoral immune mechanisms. The changes in surface antibody titres in these Ig classes were, therefore, studied before and after vaccination with a skin pathogen. Aqueous skin washings and sera were collected from Ayrshire steers prior to and following intradermal vaccination with motile zoosproes of *Dermatophilus congolensis*. The washings on each occasion were collected from the shaved skin of the back, filtered, concentrated and stored along with the sera at −20°C. Antibody titres in both were determined by enzyme-linked immunosorbent assay (ELISA) using specific antisera. Ig concentrations were estimated by radial immunodiffusion. Vaccination stimulated an increase in serum IgG antibody to *D. congolensis* but had no effect on IgM. A small decrease in the IgA titre occurred. In contrast, raised titres were found in all three Ig classes in the washings. There was, however, no difference in the amount of IgA present either in serum or skin washings after vaccination. The results demonstrate that antibody reactions on the skin in response to intradermal vaccination differ from those in the blood. They suggest that local antibody production or concentration occurs in the epidermis and support the concept of a specific antibody defence system at the skin surface. Vaccines against dermatophilosis are most effective when given intradermally and it may be that administration of antigen into the skin stimulates local antibody production more effectively. Further studies of the routes of vaccination against skin diseases are required.

### (14) A mouse model for demonstrating the protective effect of antibiotic producing skin bacteria

D.H.LLOYD, W.C.NOBLE AND S.APPIA
*Dermatology Unit, Royal Veterinary College and Department of Bacteriology, Institute of Dermatology, London*

Skin bacteria are known to produce antibiotics with a potent inhibitory effect on the growth of pathogens such as *Staphylococcus aureus, in vitro*. It has been suggested that they may also have a protective effect when established on the skin, but it has not been possible to demonstrate this *in vivo*.

A mouse model for demonstrating their protective effect was therefore developed using the skin pathogen, *Dermatophilus congolensis*. *D. congolensis* is sensitive to most conventional antibiotics and causes well-defined lesions on mammalian skin. A strain which is highly sensitive to antibiotic produced by two *Staph. epidermidis* isolates was selected as model pathogen.

White mice were clipped over the lumbo-sacral region and the skin washed with ether. Suspensions in cream of the antibiotic producers were inoculated onto the skin and aqueous suspensions of *Dermatophilus* applied after an interval of 10 min. Control mice were inoculated with plain cream or cream suspensions of non-antibiotic producing *Staph. aureus*. The skin response at 48 h showed that the level of infection on mice inoculated with the antibiotic producing organisms was reduced by approximately 50% or more compared with controls.

The results indicate that antibiotic production by skin micro-organisms can have a defensive function against potential skin pathogens. The possibility of biological control of skin diseases using such organisms must now be seriously considered.

### (15) The cutaneous reactions induced by staphylococcal protein A

MARION I. WHITE
*Institute of Dermatology, Lisle Street, London*

It is well known that *Staphylococcus aureus* may be recovered in large numbers both from the normal skin and from lesions of atopic eczema. Evidence has been presented that a bacterial count in excess of $10^6$ organisms/cm$^2$ is an indication that the bacteria are aggravating the condition. In view of the therapeutic importance of these observations the host/bacteria interactions have been studied.

Using a scrub technique the high level of carriage of *S. aureus* previously reported has been confirmed in some patients admitted for hospital treatment. When levels in excess of $10^6$ organisms/cm$^2$ have been found the carriage has declined to levels $<10^2$/cm$^2$ with conventional hospital treatment which has not included topical or systemic antibiotics.

The bacteria isolated from these patients have been studied by phage typing and by analysis for Protein A—a cell wall component in *S. aureus* with important immunological properties.

The response to an intradermal injection of Protein A is biphasic with an immediate weal and flare. The reaction is qualitatively different in atopic subjects compared to a normal control population. In addition the histological features of eczema have been produced by patch testing with this protein. These facts may be significant in the pathogenesis of eczema.

### (16) The synergistic effect of heat and ketoconazole on the growth of *Trichophyton rubrum in vitro*

D.T.ROBERTS
*Department of Dermatology, Medical Mycology, University of Glasgow*

Ketoconazole is a new systemic antifungal agent which is broad spectrum and is active *in vitro* against dermatophytes. In addition, it is known that a fungal hyphal fragment and to a lesser extent fungal spores are particularly sensitive to heat at temperatures between 45°C and 50°C. This study demonstrates the synergistic effect of heat and ketoconazole against exposed suspension of *Trichophyton rubrum*.

A spore suspension was prepared at a concentration likely to give an easily countable result in terms of the number of colonies produced. A sample of this spore suspension was incubated in a water bath at varying temperatures between 45°C and 50°C along with a further sample containing 500 $\mu$g/ml of ketoconazole. A further sample of the suspension containing the same concentration of the drug was used as a control. Aliquots were withdrawn from each tube prior to heating and at twenty minute intervals over an hour and diluted in distilled water to $10^{-2}$. Samples of each were then plated out in duplicate on 4% malt extract agar and incubated at 28°C for around a week. The colonies were then counted.

This series of experiments shows that the fungicidal effect of the drug is enhanced by heat.

This indicates that heat at the above range of temperatures is potentially a useful adjunct to the drug treatment of chronic fungal infections of feet and nails, though this potential remains to be tried in practice.

## ANNUAL GENERAL MEETING

*Chairman* PROF. RONA M. MACKIE

## SESSION 4

*Chairman* Dr W.J.Cunliffe

### (17) Prednisone treatment of acquired cold urticaria: Dissociation of histamine release and clinical improvement

A. Kobza Black, T.M.Keahey, R.A.Eady, M.W.Greaves and G.Sibbald
*Institute of Dermatology, Homerton Grove, London*

Cold urticaria is associated with elevated histamine levels in the blood draining the affected areas. However, response to therapy with conventional antihistamines is poor. Therefore the effect of prednisone, a drug which inhibits histamine release, was determined.

Clinical response and histamine release were assessed in six subjects following cold challenge before and after therapy with oral prednisolone, 20–25 mg daily for 1–3 days.

The antecubital vein of the forearm was cannulated and venous blood samples collected before and at intervals up to 30 min after immersion of the forearm in water at 10°C for 5 min. Blood histamine levels were measured using guinea-pig ilea in a superfusion cascade bioassay system. Levels of 1·0 ng/ml could be detected.

All six patients showed a rise in histamine levels on cold challenge. After prednisone therapy all but one patient showed a suppression of histamine shown as a 52–100% reduction in the areas under the plot of venous histamine concentrations against time. Although, erythema and oedema showed little diminution in all patients studied, pruritus was improved.

Whole skin histamine content of biopsy specimens from the forearm (ng/mg dry weight) before treatment (28·4) and after prednisone treatment: before (25·6) and after (27) cold challenge showed no significant change. This was consistent with inhibition of mast cell release of histamine.

The fact that there was no correlation of clinical improvement with suppression of venous histamine release suggested that histamine may not be the principal mediator of the vascular response to cold challenge in cold urticaria.

### (18) Physical urticaria: a microscopical study to evaluate the role of the mast cell

M.P.James, R.A.J.Eady, A.R.Kennedy and D.B.Gunner
*Institute of Dermatology, Homerton Grove, London*

To evaluate the role of the mast cell in the pathogenesis of physical urticaria we studied twenty-three patients: six with cold, five solar, four pressure, three factitious, two aquagenic, two cholinergic and one with heat urticaria. Biopsies were taken from weals 5–20 min following challenge (4–8 h in pressure urticaria) and from normal skin. Semi-thin plastic sections were assessed by light microscopy: (1) for mast cell population density (Eady *et al.*, 1979, *British Journal of Dermatology*, **100,** 623); (2) mast cell degranulation; and (3) morphological changes in the dermis for comparison with histamine and compound 48/80-induced reactions (James & Eady, 1979, *Journal of Investigative Dermatology*, **75,** 290).

Mast cell population density was normal in both challenged and unchallenged skin in all types of urticaria and there was no significant difference in percentages of degranulated mast cells in challenged skin compared with either unchallenged skin or normal controls. However, the degree of degranulation varied widely between patients. Morphological changes in the dermis were variable: oedema and vascular dilatation as seen in histamine-induced weals characterized weals of cold, aquagenic, cholinergic and heat urticaria. In addition, slight leukocyte infiltration and fibrin deposition, similar to reactions following histamine infusion, occurred in solar urticaria and dermographism. In contrast, greater leukocyte infiltration and fibrin deposition occurred in pressure urticaria mimicking weals induced by compound 48/80.

While the general histological findings in different forms of physical urticaria can largely be reproduced experimentally by histamine or compound 48/80 injection, our pooled results failed to provide direct evidence for mast cell involvement. Further work is needed to compare the degree of mast cell degranulation with release of mast cell derived mediators in different patients.

### (19) Sensory responses of human skin to synthetic histamine analogues and histamine

M.G.Davies and M.W.Greaves
*St John's Hospital for Diseases of the Skin, London*

The itch-producing actions of histamine are well known. In order to investigate the nature of the histamine receptors for itch, the itch responses of human skin to synthetic analogues of histamine were studied, and compared with the response to histamine itself.

Three synthetic analogues were studied: 4-methyl histamine and Dimaprit (histamine-$H_2$ agonists) and 2-methyl histamine (histamine -$H_1$ agonist). Itch thresholds were determined on a group of sixteen healthy volunteers using the epidermal scarification method of Greaves & McDonald-Gibson (1973) (*British Medical Journal*, **iii,** 608).

Itch thresholds for 2-methyl histamine were consistently higher than for histamine itself ($P < 0{\cdot}001$). Chlorpheniramine (an $H_2$ receptor antagonist) raised the itch threshold to 2-methyl histamine and histamine significantly ($P < 0{\cdot}001$). Neither 4-methyl histamine nor Dimaprit caused itching alone or potentiated itching due to 2-methyl histamine or histamine itself.

The results suggest that histamine induced pruritus is mediated in part through the $H_1$ receptor and in part via an additional (but probably non $H_2$) mechanism.

### (20) The effects of $H_1$ and $H_2$ antagonists on epidermopoiesis and epidermal structure

R. Marks, C.Tan, P.Dykes and A.Finlay
*Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff*

Histamine is found in high concentration in experimental wounds soon after their induction during the early phase of re-epithelialization (Raekallio, 1972, *Journal of Forensic Science*, **i,** 3) and exogenous histamine has been found to influence epidermal cell production *in vitro* (Harper, Flaxman & Chopra, 1974, *Journal of Investigative Dermatology*, **62,** 384). There have been no reports on the role of histamine in the *in vivo* modulation of epidermopoiesis in man. This paper describes the effects of intracutaneous histamine on epidermal thymidine autoradiographic labelling indices (TALI) and the effects of $H_1$ and $H_2$ receptor antagonists on normal and stimulated epidermal cell production in normal human volunteers. Small increases were found on TALI from injections of up to 50 μg 16 h previously. Systemic administration of chlorpheniramine reduced epidermal TALI and mean epidermal thickness in tape stripped skin, although the combination of chlorpheniramine and cimetidine was less effective. There was also some reduction in TALI in non stimulated epidermis from both the $H_1$ and $H_2$ antagonists. It appears that histamine release may play only a small pro-epidermopoietic role, that both receptors may be involved and that there may be a role for some antihistamines in disorders in which there is heightened epidermal cell production.

## ANNUAL GUEST LECTURE

### Squamous carcinoma and papillomaviruses

Prof. W.F.H.Jarrett
*Professor of Veterinary Pathology, University of Glasgow*

## SESSION 5

*Chairman* Dr J.A.A.Hunter

### (21) Investigations into the pathogenesis of monilethrix hair fracture: electron histochemical studies

C. Gummer and R.P.R.Dawber
*Department of Dermatology, Slade Hospital, Oxford*

Previous light and scanning electron microscopic studies from this department have shown that monilethrix hair shafts have an abnormal surface structure located particularly at the narrow internodes where breakage actually occurs. This cannot be explained simply on the basis of narrow diameter alone and we postulated the existence of an intrinsic biochemical weakness in the cortex.

In order to define the cortical and cuticular defect more accurately, in this study we have investigated nodal and internodal hair structure by transmission electron microscopy using general and specific electron histochemical staining techniques and compared the results with normal hair.

At both nodal and internodal sites cortical structure was abnormal with respect to cystine residue incorporation though the changes were more pronounced at the internodes. The normal cortical whorl ('fingerprint') pattern was absent and micro- and macro-fibrillar structure was disorganized. Cortical cell membrane and pigment structures appeared normal.

Specific cuticular abnormalities were also detected, the changes also being evident mainly at internodes. The cell membranes of inner cuticular layers were thrown into numerous folds, in places showing engulfed cortical material and pigment granules—these changes indicate phagocytic activity in the prehardened follicular cuticle cells.

We have therefore confirmed the presence of an intrinsic cortical and cuticular cell abnormality in monilethrix hair of sufficient severity to explain the structural fragility so well known to clinicians.

## (22) Segmental differentiation of human dermal microvasculature: Correlative light and electron microscopic study

R.A.J.Eady and J.C.Higgins
*Institute of Dermatology, Homerton Grove, London*

While injection techniques and certain histochemical reactions can reveal spatial relationships and interconnections between dermal vessels, other methods including correlative studies by light microscopy (LM) and electron microscopy (TEM) are needed for precise identification of the different segments comprising the microvasculature.

Normal skin from many body regions in twenty-five subjects was processed for TEM using four different fixation techniques. LM of serial semi-thin sections was used to determine: (1) spatial relationships of the vessels; (2) their origin and termination; (3) characteristics of the vascular wall; and (4) cross-sectional measurements of vessel diameter. Ultrastructural observations were made on cellular and extracellular components of the vessel wall. Nine distinct segments could be identified in the superficial vascular network and papillary dermis. Analysis of variance of the vessel diameter measurements revealed that the variance within each segment was not significant but that there was a significant difference ($P = 0{\cdot}001$) among the different segments. There was also a difference ($P = 0{\cdot}001$) due to method of fixation.

TEM confirmed earlier work that vascular basal lamina differences are most useful for discriminating between arterial and venous vessels. By contrast to other studies, however, closed fenestrations were regularly observed at the tips of subepidermal capillary loops.

## (23) Scarring after cryosurgery: a morphological study

J.P.Shepherd*, R.P.R.Dawber and C.Gummer*
**Department of Oral Surgery, Oxford, and *Department of Dermatology, Slade Hospital, Oxford*

It is frequently stated that cryosurgery causes less scarring than other forms of destructive treatment. In this study we have attempted to quantify this by observing the effects of five different freeze-thaw sequences on the dermis of the pig over a 6-month period.

A liquid nitrogen spray was used, and collagen fibril structure, fibre architecture, dermal thickness and wound contraction were studied by means of light microscopy (histochemistry) and scanning and transmission electron microscopy. Distortion of the tattooed skin fields was taken as an index of wound contraction, and dermal thickness was calculated from measurements of vertical skin sections prepared for light microscopy.

Collagen and elastic fibres demonstrated high resistance to cryosurgical injury. Cellular degeneration was universal after the most severe injury, but limited to superficial areas after other freeze-thaw sequences. Different cell types varied in susceptibility to injury. Cellular recolonization occurred in the dermis after 'clinical-type' injury, but full-thickness necrosis secondary to vascular phenomena supervened when injury was severe. Significant dermal thickening ($P < 0{\cdot}05$) was present at three weeks where full-thickness injury did not occur, and a decrease in thickness then commenced which was continuing at six months, when significant thinning was a feature after 2 × 30s and 1 × 1 min cycles.

It was concluded that considerable scarring followed gross cryosurgical injury to normal skin but that maintenance of fibrous architecture was responsible for lack of scarring after 2 × 1, 1 × 1 min, 2 × 30 and 1 × 30 s freeze-thaw cycles.

## POSTERS

### (1) The chorioallantoic membrane of the embryonic chick as an assay for angiogenic factors

A.T.Stockley
*The Slade Hospital, Oxford*

This project was set up to look into the possibility of developing the chick chorioallantoic membrane (CAM) as an assay for epidermal angiogenic factors (EAF) derived from normal, stripped and psoriatic skin. Although the preparation has been used to assay tumour angiogenic factors (TAF) many times since 1969 (Gittenman & Luell, 1969, *Proceedings of the American Association for Cancer Research*, **10**, 29), the interpretation of the results obtained has recently been challenged (Jacob *et al.*, 1978, *Experimental Pathology*, **58**, 241) as dramatic angiogenesis comparable in intensity to that produced by TAF may be produced by the filter paper used to apply the factor to the CAM.

A pilot study was therefore initiated at the Slade Hospital to investigate the suitability of the preparation for a one-man research team in a basically clinical department, and to find a carrier material for angiogenic factor that has no intrinsic activity.

The protocol used in Aberdeen (A. Fraser, personal communication) was adopted and simplified, and it was found that thirty-six eggs per week could be prepared and studied by one worker. Serial photography was facilitated by injecting Indian ink through a glass micropipette. Many absorbent materials, such as might be used to apply TAF, were found to incite a non-specific inflammatory response which was associated with angiogenesis. However, it was found that a glass ring could be used to contain a pool of fluid without inciting angiogenesis, and it is suggested that this may be a more suitable vehicle.

Other aspects of non-specific stimulation of angiogenesis are discussed and diagrams and photographs are included in the presentation.

### (2) Epidermal and corneocyte size changes with age.

R.Marks
*Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff*

Routine light microscopy reveals that the epidermis from elderly subjects appears thinner than that from young or mature subjects. However, despite their biological importance and practical relevance in the study of the effects of drugs on the epidermis, there are scant quantitative data on age related epidermal changes in the literature. The aims of this study have been (a) to characterize and quantify age changes in the structure and dimensions of the epidermis and keratinocytes and (b) to relate changes in keratinocyte size with changes in corneocyte dimensions in order to learn about changes in the process of differentiation with increasing age. Measurements were made by projection techniques from carefully prepared histological sections and smears of corneocytes according to published methods (Holt & Marks, 1976, *British Medical Journal*, **1**, 496; Hölzle & Plewig, 1977, *Journal of Investigative Dermatology*, **68**, 350). There was decrease in epidermal thickness with increasing age both in absolute terms ($\mu$) and in cell numbers (MET) though the decrease in absolute terms was more striking. When mean keratinocyte height (MKh) was plotted as a function of age a statistically significant positive linear correlation was found. Figures are given for arm skin but there were similar findings from thigh, abdomen and facial skin. Corneocyte area was found to increase with age in all sites. The apparent paradox of decreased keratinocyte dimensions and increasing corneocyte size with increasing age probably signifies decreased stratum corneum transit time and may partially explain the altered physiology of the horny layer in old age.

## (3) The analysis of human epidermal proteins by SDS-polyacrylamide gradient gel electrophoresis

P.E.Bowden and W.J.Cunliffe
*Department of Dermatology, The General Infirmary, Leeds*

The present study was initiated to develop a small scale technique for the extraction and analysis of proteins involved in keratinization with a view to investigating the pilosebaceous duct. This preliminary communication involves application of the method to epidermal proteins, abnormalities of which have been found in psoriasis.

Epidermis was removed from human scalp and foreskin by three techniques and 'prekeratin' was isolated by modifying the procedure of Skerrow (1977, *Biochemica et Biophysica Acta*, **494**, 447). Stratum corneum, callus, psoriatic and ichthyotic scale were extracted with Tris-urea-mercaptoethanol according to Skerrow & Hunter (1978, *Biochemica et Biophysica Acta*, **515**, 474). All samples were analysed by SDS-gradient slab gel electrophoresis (Laemmli, 1970, *Nature*, **227**, 680; Lambin, 1978, *Analytical Biochemistry*, **85**, 114).

No qualitative differences were found in the prekeratin polypeptides from epidermis prepared by the three different methods. Prekeratin from scalp and foreskin were similar and were characterised by four major (molecular weights:- 69,000; 60,000; 56,000; 50,000) and two minor (66,000 and 45,000) polypeptide chains.

The polypeptide chain composition of normal stratum corneum and callus differed both from prekeratin and from each other. Ichthyotic scale resembled normal stratum corneum in polypeptide chain composition while psoriatic scale differed from all other extracts and was characterized by a large number of polypeptide chains over a wide range of molecular weights (13,000–150,000).

These results are at variance with other reported work and will be discussed in relation to terminal differentiation of the keratinocyte and heterogeneity of the $\alpha$-fibrous proteins.

## (4) Comparative surface architecture of human hair in affected and unaffected skin in psoriatic subjects

J.M.Riggott and E.H.Wyatt
*University of Hull and Hull Royal Infirmary*

The purpose of the study is to show a difference in epidermal activity between affected and unaffected areas in psoriasis as measured by hair morphology. The authors have already shown that there are sex, age and site differences in cuticular scale size in hairs from subjects unaffected by any skin disease.

Using a method previously described based on a statistical analysis of the numbers of overlapping cuticular scales per unit length from scanning electron micrographs of hairs, this method has been extended to appraise the significance of such measurements in psoriatic patients using samples from affected and unaffected areas of the same patient. It is shown here that in patients with psoriasis, the cuticular scale size of hairs from affected areas is greater than that in hairs from unaffected areas (females $P < 0{\cdot}01$; males $P < 0{\cdot}01$). The diameters of hair measured from the same scanning electron micrographs have shown a significant difference between the sexes, and also hairs cut from affected areas are significantly different in their diameter from hairs cut from unaffected areas in psoriatic patients, males having a mean difference of 6·1 μm, females having a mean difference of 6·7 μm.

It is suggested that hair diameter and cuticular scale size are a measurable parameter of the keratinising potential of the hair follicle in psoriasis, and can be used to demonstrate differences in epidermal turnover now regarded as being characteristic of the disease.

### (5) A method for the *in vivo* study of human superficial skin lymphatics

D.Murray
*Institute of Dermatology, Lisle Street, London*

A neglected method (Hudack & McMaster, 1933, *Journal of Experimental Medicine*, 57) for the *in vivo* study of human superficial skin lymphatics has been reassessed and modified.

An Olympus OM-2 automatic camera with fully automatic flash system and motor drive was fitted to a WILD M8 zoom stereo microscope giving a variable magnification of ×6 to ×50 at a working distance of 8 cm. Disulphine Blue (I.C.I.) 6·2 w/v was diluted in normal saline and injected superficially into the skin through a 27 gauge butterfly needle connected via a polythene cannula to an electric syringe pump. The skin surface is flooded with oil to allow better definition and the infusion started. The rapidly developing network of superficial lymphatics is serially photographed using Tri-X B.W. film. An initial study of the anatomical patterns and organization of normal adult human skin will be presented. Later studies will include the skin in pathological states together with an *in vivo* assessment of lymphatic function using the same dye technique.

### (6) Changes of enzyme activities in suction blister fluid from skin subjected to graded thermal injury

M.C.Middleton and R.Hasmall
*I.C.I. Limited, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire*

If skin responds to cellular injury in a manner analogous to that of other organs, then release from the damaged cells of intracellular enzymes representative of different cellular compartments could indicate the severity of the cutaneous damage. For example, mild injury to the liver results in release of cytosolic enzymes into the plasma, moderate injury in release of cytosolic and mitochondrial enzymes and severe injury in release of cytosolic, mitochondrial and lysosomal enzymes.

We have previously demonstrated that enzyme activities are increased in suction blister fluid from chemically injured skin and that the increases are the result of local and not systemic injury.

We, therefore, subjected skin of anaesthetized rats to injury of increasing severity by placing one clipped dorsal flank in contact with a hot-plate for 30 s at temperatures of 50–65°C. Suction blisters were then raised on the damaged skin (animals anaesthetized throughout). Blister fluid was collected from control and treated flanks 3 h after injury and the activity of cytosolic, mitochondrial and lysosomal enzymes determined.

After mild or moderate thermal injury there were substantial increases in cytosolic enzymes in the blister fluid but there were concomitant although smaller increases in mitochondrial and lysosomal enzymes. After severe injury (freezing and thawing) lysosomal enzymes were markedly increased.

Skin injury results in release of intracellular enzymes from the damaged tissue, a response similar to that observed in other organs, but the pattern of release resulting from injury of increasing severity differs.

## WORKSHOPS

### Saturday 19 January

| | |
|---|---|
| (1) Photobiology | Dr W. Frain-Bell<br>Dr B.E. Johnson |
| (2) Bullous Disease | Dr M. Black<br>Dr C. Skerrow |
| (3) Endocrine Function of Skin | Prof. J. Ebling<br>Dr R. Marks |
| (4) What's New in Psoriasis | Dr H. Baker<br>Dr D. Skerrow |

*British Journal of Dermatology* (1980) **102,** 743.

*Society Proceedings*

# Proceedings of the 216th Meeting of the Netherlands Society for Dermatology and Venereology, Rotterdam, October 1979

PRESIDENT: DR M.J.WOERDEMAN
HOST: PROFESSOR E.STOLZ

## CASE REPORTS

*Department of Dermatology and Venereology, Academic Hospital, Rotterdam*

### Erythropoietic protoporphyria

A.F.D.Smit

Three brothers and one sister: Patient A: male, aged 48 years; Patient B: male, aged 49 years; Patient C: male, aged 41 years; Patient D: female, aged 52 years.

*History.* Patient A: as long as the patient can remember he has been troubled by a burning, painful and itchy feeling as soon as he exposes himself to the sun. At the same time swelling and blue-red discoloration of the skin occur. When sitting in the sun behind glass, he has the same complaints. He is affected much more when the wind is in the east. Artificial light presents no problems. Contralume cream (a sunscreening agent) did not improve the symptoms. In all, four brothers and sisters have in a greater or less degree the same complaints. One sister lives in South Africa, the others are present (Patients B, C and D).

*Dermatological examination.* The skin on the back of the hands, on the metacarpophalangeal joints, is thickened and coarsened. The face is light-red. In Patients B and C the abnormalities are most evident. These patients show, in addition, some small vertical furrows on the upper lip. Patient D has only slight abnormalities.

*Histology.* Patient A, biopsy forehead: the biopsy is covered with a slightly papillomatous, slightly acanthotic and hyperkeratotic epidermis. Skin appendages occur mainly in the form of sweat-glands. Around the slightly widened vessels in the superficial and middle dermis, homogeneous, strongly PAS-positive deposits are found. The endothelium of these vessels is generally slightly swollen. A very slight round cell infiltrate, mainly perivascular, is present.

*Laboratory data (Patient A).* Light tests: MED 300 nm $= 3 \times 10^4$ W/m$^2$; normal value: $16 \pm 6 \times 10^4$ W/m$^2$. MED 320–700 nm $= 63 \times 10^4$ W/m$^2$; normal value $> 300 \times 10^4$ W/m$^2$. MED 400 nm $= 91 \times 10^4$ W/m$^2$; normal value $> 300 \times 10^4$ W/m$^2$. MED 450 nm $= 100 \times 10^4$ W/m$^2$; normal value $> 500 \times 10^4$ W/m$^2$. Fluorocytes: strongly positive. Porphyrin: Patient A 7500 nmol/100 ml erythrocytes (normal 150); Patient B 7900; Patient C 9900; Patient D 7000. The urine did not fluoresce and was of a normal colour. Protoporphyrins: Patient A 780 nmol/gram dry weight of faeces; Patient B 740; Patient C 990; Patient O 960. Coproporphyrins: traces in all patients. Urine: values within normal ranges.

*Treatment.* 60–180 mg beta-carotene/daily with no real benefit.

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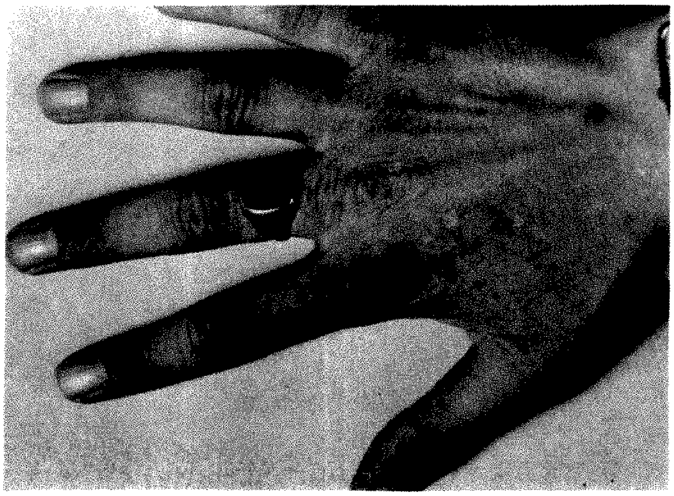

FIGURE 1. Erythropoietic protoporphyria.

## Necrolytic migratory erythema

M.S. DEN BOER AND E. STOLZ

Male, aged 56 years.

*History.* He first attended in May 1979 for an itching skin eruption since September 1978, which had begun on the legs as red spots. Centrally, blister formation occurred. The spots spread over the entire body. The eruption was accompanied by a general feeling of depression. In 1975 he had been under psychiatric treatment for depression.

*Dermatological examination.* Annular, erythematous lesions were observed on the legs, lower arms, lower abdomen, buttocks and groin. Some of these presented central bullae or erosions with crusts. The lesions were confluent predominantly in the groin and on the lower abdomen; they healed after approximately 10 days, leaving residual pigmentation. This was followed by new lesions in other areas. On the face, brown-red crusts were observed around the nose extending into the bearded area. The tongue was smooth and red, and the corners of the mouth were sore.

*Examination.* He was slow reacting, depressed and had a cachectic appearance.

*Histology.* From a skin biopsy from left knee the epidermis presents irregular acanthosis. In the upper layers there is nuclear pyknosis and homogenisation of the cytoplasm. There is local spongiosis. In the upper dermis, there is a round-cell inflammatory infiltrate extending into the epidermis.

*Laboratory data.* ESR 10 mm/hr, Hb 7·9 mmol/l. Leukocytes 5·7 × $10^9$/l. Kidney and liver function-tests normal. Total serum protein 61·3 g/l, albumin 40·5 g/l, α1 glob. 1·8 g/l, α2 glob. 6·7 g/l, β glob. 6·1 g/l, γ glob. 6·1 g/l. Fe: 18·3 μmol/l, TIBC 60 μmol/l. Insulin blood level: 28 mE/l. Blood zinc: 83 μmol/l (normal value 76–140 mmol/l). Blood sugar curve: values rising to 14·2 mmol/l. Arteriography of the pancreas: large space occupying process in the tail of the pancreas, with a diameter of ±6 cm. Glycogen content in blood: 3000 pg/ml (normal value 90 pg/ml). Plasma amino-acids were reduced to 50%.

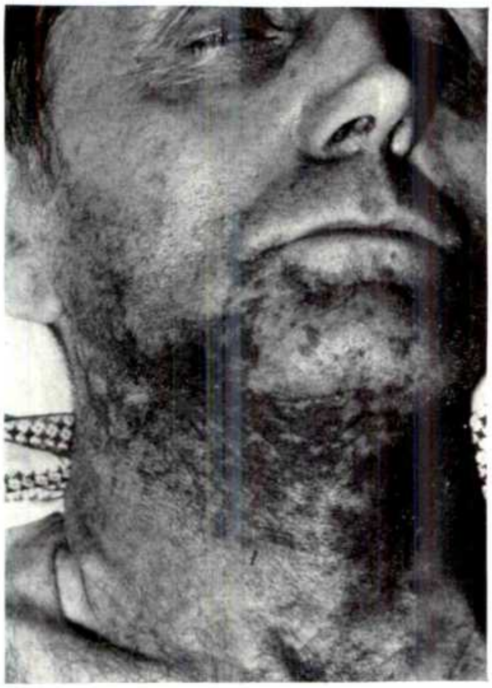

FIGURE 2. Necrolytic migratory erythema.

*Course.* The patient was transferred to the Surgical Department. At laparotomy a large tumour was found in the pancreas, with liver metastases. Hemipancreatectomy and splenectomy were performed. Histological examination of the pancreas showed a very extensive invasive tumour in a surrounding pseudocapsule. The tumour tissue showed a trabecular character and a tendency to form tubular structures. The spleen was engorged. The liver metastases presented the same picture as the pancreatic tumour. Immunofluorescence of the tumour with antiserum induced in rabbits against glucagon showed a granular cytoplasmic fluorescence. The patient died quite suddenly 9 days after the operation. The most probable cause of death was pulmonary embolism.

## Sarcoidosis of the skin (nodular form), mucous membranes of nose and mouth, and lungs

M.S.DEN BOER

Female, aged 34 years.

*History.* She has been suffering from a skin eruption for 6 months; this started with a nodule on the inside of the lower lip. Later, the eruption extended over the entire body. Also she has been complaining for 3 months of a stuffed-up nose. The lesions sometimes itch and are painful when pressed. She is a negroid and originally comes from Cameroon; she has been living in Holland for 10 years.

*Dermatological examination.* Skin: on the arms, legs and trunk there are multiple cutaneous and subcutaneous nodules. They vary in size, are not attached to the underlying tissues and are firm. The superficially situated nodules sometimes present excoriations and depigmentation.

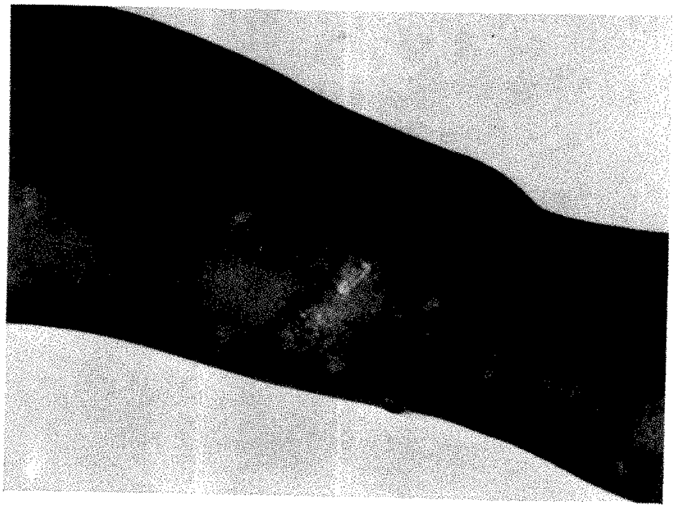

FIGURE 3. Sarcoidosis.

Mucous membranes: nodules can be seen on the palpebral conjunctiva of the right lower lid, and on the inner side of the lips and the palate.

In the nose multiple nodules are visible on the septum and on both sides on the conchae.

*Histology.* Nodule on the right upper arm: in the dermis, situated deeply, as well as directly under the epidermis, there are granulomas varying in size, consisting of histiocyte-like cells, lymphocytes and multinuclear giant cells. Some granulomas are situated around the adnexae; others are lying in the dermis.

There is no central necrosis. Ziehl-Neelsen, Wade-Fite and Whartin stains are negative.

Nodule from the nose: focal nests of epithelioid cells which have a tendency to form granulomas.

*Laboratory data.* ESR 8 mm/h, Hb 7·9 mmol/l. Leukocytes: $4{\cdot}4 \times 10^9$/l. Syphilis serology, dab-preparation of skin biopsy for acid-fast bacilli and Ziehl-Neelsen smear test of material from nose: negative. Chest X-ray and tomography showed hilar gland enlargement on both sides. X-rays of hands and feet: moderate degenerative abnormalities in both first metatarsophalangeal joints. Mantoux test: negative (1 Tu and 10 Tu). Kveim test: not carried out.

*Therapy.* Due to the extent of the skin lesions and the abnormalities of the nasal mucous membrane, the patient is being treated with prednisone in an initial dose of 40 mg daily.

## Telangiectasia eruptiva macularis perstans and systemic mastocytosis

K.H.Tjiam, R.Ouwendijk and E.Stolz

Male, aged 62 years.

*History.* Since 1961 he has suffered from a nagging pain in the neck and the low lumbar region. Since 1965 he has suffered from itching attacks several times a day. These attacks occur especially after alcohol, certain medicines, sunlight, and showers. After drinking two glasses of wine, after taking certain medicines and also spontaneously, he also experiences a red, warm face, which persists for a period of 15–30 min. Such attacks occur several times a week. During the last few years the complaints have increased progressively.

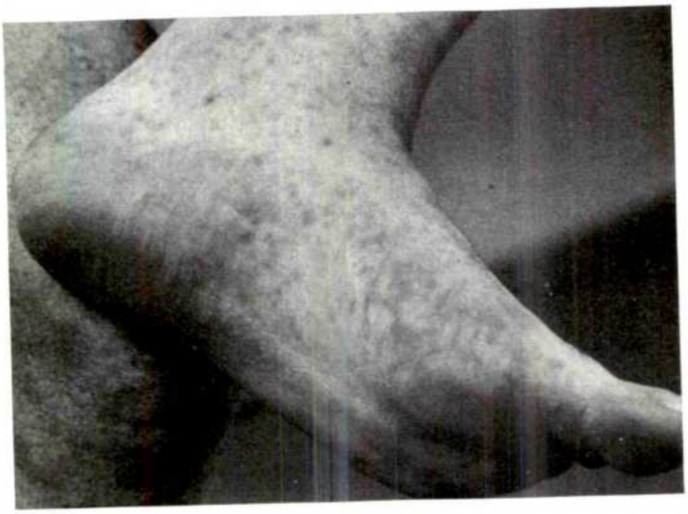

FIGURE 4. Telangiectasia eruptiva macularis perstans.

*Dermatological examination.* On the trunk and extremities, telangiectatic macules are visible. It is possible, with pressure, to empty the telangiectases. On the backs of the legs, more on the left than on the right leg, and on the knees, the telangiectases are of a fiercer red, and are papular. When pressing these papules, the erythema disappears, but on the back of the upper legs and on the knees, a brown-yellow pigmentation remains at the site of the papules. On the palms and soles, an extensive telangiectatic erythema is visible. The Darier sign is positive.

*General examination.* He has a red face. The liver is two fingers palpable, firm, not painful. The spleen is one finger palpable, firm, not painful. There is slight limitation of movement of the cervical and lumbar spine with low lumbar tenderness.

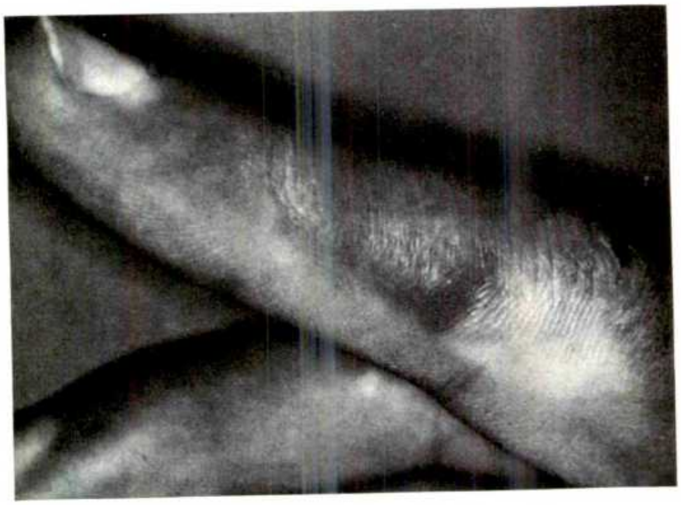

FIGURE 5. Lymphangioma-like Kaposi's sarcoma.

*Histology.* A fresh lesion from the back of the thigh shows slight acanthosis. The superficial dermis shows some oedema especially around the vessels and a slight round cell infiltrate with some eosinophil granulocytes. Toluidine blue staining shows a large number of mast cells around these vessels. The vessels in the superficial dermis are generally dilated.

The liver biopsy, bone marrow and material obtained by bone drilling, all show an increased number of mast cells.

*Laboratory investigations.* Histamine excretion in urine: 360–415 mg/24 h (normal up to 60 mg/24 h). Histamine in plasma: 5·4 ng/ml (normal). Cholesterol 3·7 mmol/l (normal 3·8–8 mmol/l). X-ray of spinal column: degenerative abnormalities and local thickening of bone structure. X-ray of pelvis: osteoblastic condensations of the heads of both femurs and os pubis.

*Therapy and course.* Initially he was treated with H1 and H2 blockers (hydroxyzine bihydrochloride and cimetidine) without result. He was then treated with oral sodium cromoglycate likewise without any therapeutic result. At present chlorambucil 4 mg/daily oral is under evaluation.

## Lymphangioma-like Kaposi's sarcoma

I. VERMEIDEN AND V.D. VUZEVSKI

Male, aged 30 years.

*History.* In February 1978, the patient, a Moroccan male, visited the dermatology policlinic with painful, blue-red areas on the fingers of both hands and on the inner sides of his feet, which had been present for several months. Recently these areas had increased in number and size.

*Dermatological examination.* Livid plaques were observed on the dorsal aspect of Digits I, II, III and V of the left hand, digits IV and V of the right hand and at the level of the medial malleoli on both feet. On palpation these plaques felt soft and warm. With diascopy the livid colour did not disappear.

*Histology.* The epidermis is normal. Tumour tissue appears in all layers of the dermis. The tumour consists of lymph vessels lined by endothelial cells and of solid masses composed of oval or coil-shaped cells. Narrow split-like spaces exist between the collagen bundles (collagen dissection). The endothelial cells lining the vessels are larger and more numerous than in normal lymph vessels, but they do not present nuclear atypy. The solid tumour masses consist of coil-shaped or oval cells, with moderate nuclear pleomorphism. In some areas a number of erythrocytes can be seen scattered in the tumour mass, macrophages charged with haemosiderin and a moderate inflammatory infiltrate.

*Investigations.* Blood chemical analyses, X-rays of the bones, and liver, spleen, brain and total body scans showed no visible abnormalities.

*Therapy.* Treatment consisted of radiation with 25 MEV 400 rad in waterbath 3 times a week, for 2 weeks. The result of the radiation is satisfactory. The plaques have decreased in size considerably and the patient suffers less pain than before.

### REFERENCE

GANGE, R.W. & WILSON JONES, E. (1979) Lymphangioma-like Kaposi's sarcoma. *British Journal of Dermatology*, 100, 327.

## Leprosy (borderline case)

B. DE SWAAN, H.E. MENKE AND D.L. LEIKER

Female, aged 44 years.

*History*. An Indonesian woman who has been living in Holland since 1977. In 1974, when in Indonesia, a small red skin eruption became apparent on the left leg, which did not increase in size. After her fourth pregnancy in July 1979, more red patches developed and spread over the entire body.

*Dermatological examination*. Extensive, rather sharply defined, partly annular, infiltrated erythematous plaques, as well as sharply defined dome-shaped erythematous lesions are localized on face, trunk and extremities. The eruption is symmetrical. No thickening of the nerves has been observed. Sensation in the lesions is not clearly disturbed.

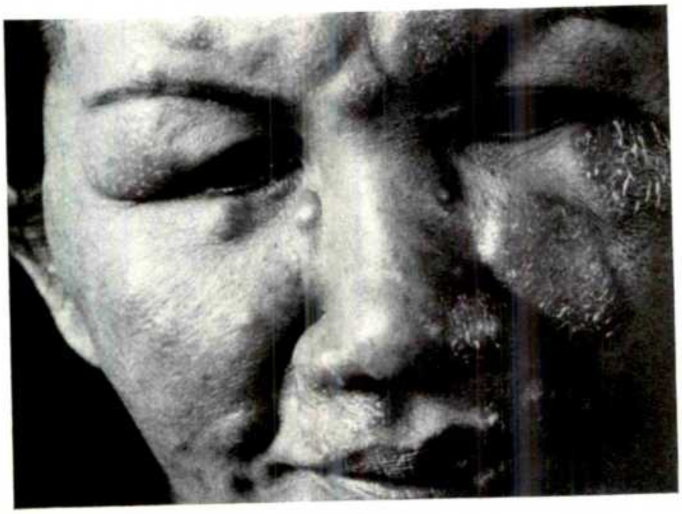

FIGURE 6. Leprosy.

*Histology*. The epidermis is normal. In the papillary and reticular dermis there are solid nests of epithelioid cells and lymphocytes. The epithelioid cells have an eosinophil cytoplasm and some have small vacuoles. Nerve branches have not been observed in this material. Wade-Fite-Staining: acid-fast bacilli present.

*Therapy*. 600 mg Rifadin and 100 mg dapsone daily.

*Comments*. In view of the large number of partly dome-shaped lesions, the tendency to symmetrical distribution, the absence of sensory disturbances and the histological findings, the patient was classified in the borderline group. Leprosy is known to exacerbate during pregnancy, but in particular after delivery and in the lactation period.

## SUMMARIES OF PAPERS

### Erythromelalgia and peripheral gangrene in thrombocythaemia

J.Michiels*, J. Lindemans*, I.Vermeiden†, J.Steketee‡, H.van Vliet§, V.D.Vuzevski¶ and J.Abels*
*Departments of *Haematology, †Dermatology and Venereology, §Neurology, Academic Hospital; Institutes of ‡Biological and Medical Physics, and ¶Pathology, Erasmus University, Rotterdam, The Netherlands*

Clinical studies were undertaken in patients with thrombocythaemia. Erythromelalgia is often the presenting symptom, but because of its unfamiliarity is frequently not recognized by the physician. Usually the patient with ischaemic symptoms secondary to thrombocythaemia is treated for occlusive vascular disease without relief. Pain can usually be treated effectively with low doses of acetyl salicyclic acid, and remission of the myeloproliferative disorder may be obtained with appropriate chemotherapy, for which busulphan usually suffices. Vacular insufficiency becomes ultimately irreversible with fairly typical histological changes. Functional abnormalities of the platelets are often evident in their propensity to aggregate spontaneously *in vitro* and by their shortened survival *in vivo*. These signs returned to normal under treatment with acetyl salicylic acid. The disease is not rare and we have been able to study more than twenty symptomatic patients.

### Non-specific hypersensitivity of the skin

L.v.d.Lugt and T.S.Veninga

*Department of Dermatology, Academic Hospital, University of Rotterdam, and Radiopathology Laboratory, University of Groningen, The Netherlands*

Albino hairless mice have been used to test the hypothesis of remnant mediators circulating in extensive non-allergic dermatitis which might be responsible for false positive reactions in a patch test series of otherwise harmless doses of irritants applied to non-affected skin. Two open patch tests were placed with 10 $\mu$l of 2% or 3% phenol solution in ether cranially or caudally on one flank with an interval of 10 days between them. Intermediately, 10 $\mu$l of 10% phenol was applied contralaterally 48 h before the second patch deposition in some of the animals. The remaining mice served as controls.

Intermediately treated animals showed in general a more pronounced reaction in the second patch as compared to the first. In six of twenty-two cases the distinction was very striking. Histological examination revealed a more extensive epidermal lesion as well as a larger granulocytic infiltrate. In controls, only modest differences were observed.

These findings favour the hypothesis that mediators of inflammation present in the circulation strengthen the effect of weak skin irritants.

### Hyperkeratosis lenticularis perstans (Flegel)

W.J.B.M.van de Staak, A.M.G.Bergers and P.Bongaarts

*Department of Dermatology, University of Nijmegen, The Netherlands*

Hyperkeratosis lenticularis perstans (HLP) is a rare, probably autosomal dominant disorder of keratinization, first described by Flegel (1958). The clinical picture of HLP consists of red-brown horny scales of irregular outline (1–5 mm in diameter) found mainly over the upper surface of the foot and the lower leg. The thighs, arms, hands and trunk are less frequently involved. In addition, one can often observe pinpoint-sized keratotic papules and pits interrupting the dermatoglyphics of palms and soles. Abnormalities of the mucosal membrane have never been described in HLP. Characteristic electron microscopic features have been reported by Frenk & Tapernoux (1974, 1976); these include lack of Odland bodies in the epidermis underlying the keratotic papule and a persistence of the desmosomal discs through the whole stratum corneum.

Recently we have seen two patients with clinically and histologically typical HLP. Both patients, however, showed additional abnormalities of the mucosal surface. Electron microscopic investigation indicated the absence of Odland bodies both in the cutaneous and in the mucosal lesions of these patients. These ultrastructural findings, both in the cutaneous and in the mucosal lesions, provide strong evidence that the mucosal abnormalities are a real component of hyperkeratosis lenticularis perstans.

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FLEGEL, H. (1958) Hyperkeratosis lenticularis perstans. *Hautarzt*, **9**, 362.
FRENK, E. & TAPERNOUX, B. (1974) Hyperkeratosis lenticularis perstans (Flegel). *Humangenetik*, **24**, 151.
FRENK, E. & TAPERNOUX, B. (1976) Hyperkeratosis lenticularis perstans (Flegel). *Dermatologica*, **153**, 253.

Abstracts of other papers will appear in the *British Journal of Venereal Diseases*.

*British Journal of Dermatology* (1980) **102**, 753.

# *Book Reviews*

**Techniques in Skin Surgery.** ERVIN EPSTEIN and ERVIN EPSTEIN, JR (1979) London: Henry Kimpton. Pp. 203, B/W illustrations, 1 col. plate. Price £12.50.

This superb monograph deserves nothing but high praise. It is of particular relevance to those dermatologists training (and trained) by the British system in which good skin surgery teaching is almost non-existent even though most departments undertake considerable amounts of minor surgery. In 200 pages—twenty-nine brief chapters—all the standard surgical, chemosurgical and cryosurgical techniques are described in a concise, didactic and very practical style. The sections on incision and suture methods are highly commended since they describe many ostensibly complicated surgical techniques such as Z- T- and M- plasty and H- closure in such a way as to take the 'magic' out of the simpler end of plastic surgery. The reviewer's patients have already benefited! R.P.R. DAWBER

**Practical Pediatric Dermatology.** WILLIAM L. WESTON (1979) Boston: Little, Brown & Co. Pp. 361, B/W illustrations. Price £10.95.

This is a well produced easily read text-book. It packs a lot of practical information into its 345 pages of text, which are divided into seventeen chapters, each ending with relevant references. It is intended for those engaged in primarily the care of children, and they will find it extremely useful.

Each disease is treated in a similar way, dealing with clinical features, differential diagnosis, pathogenesis, treatment, patient education, and follow-up visits.

The text is enhanced by sixty-seven tables, and twenty-three line drawings; there are also 229 black and white photographs, but unfortunately some of these are of a very poor standard; this is possibly because they have been taken from colour slides.

There are a few surprising statements and also some surprising omissions, e.g. the absence of dithranol for psoriasis.

Overall this is an extremely readable and useful book.

JOHN E. WHITE

**Morphological Dermatology. A Study of the Living Gross Pathology of the Skin.** ROBERT JACKSON (1979) Springfield. Illinois: Charles C. Thomas. Pp. 338, 86 illustrations. Price $32.50.

This book is designed as an aid to diagnosis of skin disorders considering only their gross morphology. The author has, over the last few years, stressed in the *Archives of Dermatology* the importance of morphology in arriving at a correct diagnosis and his feeling that the development of other methods of examination, including histology, immunofluorescence, etc, vital though they be, may have appeared to render close visual examination of less importance than in the past.

Skin diseases are grouped according to the morphological changes occurring. Unfortunately, the overlap between clinical manifestations is so frequent that it is obviously difficult to group conditions in this way. One of many examples of this is the inclusion of vesicular fungus infections under eczema, a contrived association which surely cannot be justified on morphological grounds.

There are a number of mis-spellings, e.g. Fogo Solvagem and Brachial Sinus. There are also some errors of fact such as the description of the halo naevus as having 'a light coloured anaemic halo', 'carbon dioxide forms carboxyhaemoglobin' and the extrusion of 'sebaceous material' from a wen. Epidermoid and pilar cysts are not mentioned as such, or differentiated.

It is difficult to decide on the group of readers for whom the book is intended. It mentions many quite rare diseases so that it is too comprehensive for the medical student and the diseases are rather superficially dealt with without any mention of the processes leading to the morphological change so that it is not really suitable for the dermatologist in training. The trained dermatologist would, I am sure, be familiar with the morphological features that are mentioned and would rarely find it necessary to refer to this book.

The teacher of dermatology may perhaps get some points in descriptive morphology that could help him in the demonstration of skin disease.

The book is a worthy attempt to base diagnosis entirely on morphology but it fails because there is no explanation of the underlying pathology which leads to the morphological manifestations seen in the skin. Pathogenetic mechanisms are surely a more logical basis for grouping all diseases including those of the skin.

R.H.MEARA

**Microbial Disease: The Use of the Laboratory in Diagnosis, Therapy and Control.** D.A.J. LYRRELL, I.PHILLIPS, C.S.GOODWIN and R.BLOWERS (1979) London: Edward Arnold. Pp. 340. Price £16.

The authors refer to themselves as having worked at, indeed are still working at, the interface between laboratory and clinical medicine. They regard microbiology as having been taught too much as a laboratory discipline and too little as an integral part of clinical practice. Their book is an attempt to restore the balance.

The book is organized in three sections covering principles of microbiological diagnosis and antibiotic therapy, diseases of the individual body systems, and diseases involving more than one system. For the clinician, this book will be valuable in indicating which pathogens may reasonably be expected and when that pathogen will normally be sensitive to certain antibiotics. The specimens most likely to yield good diagnostic material are also given. Inevitably it will prove of greatest value in areas peripheral to the clinicians' norm. For the microbiologist the chief value will lie in suggesting potential pathogens or tests likely to reveal pathological changes in patients for whom no firm diagnosis has yet been made. The authors stress the value of a good clinical diagnosis which may then be confirmed by appropriate laboratory tests.

By using a crisp style the authors have contained the subject matter in a reasonable number of pages; nevertheless the book remains readable. This and the value of its contents will serve to keep it in constant use. Guard your copy, others will want it.

W.C.NOBLE

Re: *Microbial Disease* by Tyrrell, Phillips, Goodwin and Blowers.
Owing to a potentially dangerous error on page 279, the publishers have asked that all copies of the book should be returned for correction to: Mr M.Husk, Trade Department, Edward Arnold (Publishers) Limited, Woodlands Park Avenue, Woodlands Park, Maidenhead, Berks.

**Reactions of the Skin to Cosmetic and Toiletry Products.** CONSUMERS' ASSOCIATION (1979) Caxton Hill, Hertford: Consumers' Association. Pp. 154. Price £50.00.

This report from the Consumers' Association details the results of a large scale survey undertaken to establish the incidence, in the general population, of adverse skin reactions to cosmetics and toiletries. The project was divided into three stages. In the first, the Omnibus survey, 11,062 people were interviewed and questioned as to whether they thought that they had reacted adversely to a cosmetic or toilet preparation in the previous year; 12% made such a claim. From the reactors further information was obtained by means of a postal questionnaire and the products most frequently incriminated were deodorants and anti-perspirants (25%), eye-make-up (14%) and soap (12%).

In the third stage one particular parliamentary constituency was studied. Here 1022 people were interviewed; eighty-five (8%) reported a reaction and of these forty-four were patch tested by a dermatologist. The patch test reactions were allergic in eleven, irritant in twenty-three and not a cosmetic problem in ten. The allergic reactions were to deodorants (2), hair dye (1), lipstick (3), skin creams (4) and perfume (1).

In the dicussion these patch test results are extrapolated to the general population but forty-four subjects with eleven relevant patch test reactions are not sufficient data on which to base a population survey.

The report is bound in a loose leaf folder, it comprises fifty-three pages of text, thirty pages of appendices and seventy-one pages of tables.

E.CRONIN

**Pigment Cell. Volume 4: Biologic Basis of Pigmentation. Volume 5: Pathophysiology of Melanocytes.** Proceedings of the 10th International Pigment Cell Conference, Cambridge, Massachussets, 1977. Parts I and II. Edited by S.N.KLAUS (1979) Basel; S.Karger. Pp. 358; Price S.Fr. 159. (Vol. 4) Pp. 318; Price S.Fr. 139. (Vol. 5.)

Ten international pigment cell conferences have been held since 1946 and these two books summarize the proceedings of the last conference which took place in Cambridge, Massachussets, U.S.A., in 1977. Few people with an interest in pigment cell research would doubt that these meetings have been very successful, and have provided a remarkable opportunity for scientists and physicians from different biological and medical disciplines to get together and discuss common problems. It is also lucky for those who could not attend that their proceedings have been published in detail.

The eighty-six papers summarize much of the pigment cell and melanoma research carried out between 1975 and 1977. There should be something in one of the volumes to interest every pigment cell biologist. For example, the way in which tyrosinase is transported from the Golgi complex to the earliest premelanosomal matrix has interested me for a number of years; it came as quite a surprise that there may be much to learn from research on the goldfish and adult leaf frog. In fact the mature melanosome in the melanophore of the adult leaf frog looks quite similar to the giant melanosome seen in some pigmentary disorders of the human. Of course this subject is not everybody's cup of tea, but it is a good example of how interdisciplinary liaison can help to unravel problems.

Although many of the contributions have already been published in similar form, the volumes provide a useful starting off point for chasing reasonably current views and references. As usual, I enjoyed the clear description of the detection of phaeomelanin by Rorsman and his colleagues. Time will tell whether there will be a practical application for the depigmenting dicarboxylic acids produced by the enzyme action of *pityrosporum orbiculare* (Nazzaro-Porro and co-workers). Meanwhile it is good to know a scientific reason for such a striking clinical phenomenon.

There are thirty-one papers on malignant melanoma in Volume 5. Overall I detected rather less emphasis than recently on immunological studies and more on the effects of exogenous agents on melanoma cell growth and the biochemical characterization of melanomas including the measurement of serum tyrosinase and urinary melanogens in melanoma patients. Again this section should provide many helpful references.

Two chapters deserve special mention. Dr Lerner's reminiscences were most entertaining. He points out that aging academics need not view the future with total gloom. If they survive three score and ten years they might even contribute to the second peak of publications illustrated in the graphs where the average number of contributions by scientists during 5 year intervals is plotted against their age. The keynote address given by the late Dr I.W.Whimster provides a lasting reminder of his unusual but most logical thought process. How we will all miss these rare blends of science and art, even if many of us approached them with a degree of intellectual masochism.

It is inevitable that these volumes contain one or two rather light weight efforts but they do not detract from the general high standard. It would, however, be helpful to have formal summaries of each contribution when the proceedings of the 11th International Pigment Cell Conference are published. If much of the content in these books is like plonk—have had plenty before but continues to be interesting, enjoyable and a little bit different—then there is also quite enough good claret here for the connoisseur.

J.A.A.HUNTER

*British Journal of Dermatology* (1980) **102**, 757.

# British Association of Dermatologists

## WILLAN LIBRARY BULLETIN V

During the past year the Willan Library has received several gifts, which are greatly appreciated. These gifts have included copies of some of the books which the late Ian Whimster considered had had the greatest influence on his scientific development: they have been presented to the library in his memory, by Dr and Mrs Robert Jackson of Ottawa, Canada. These books and those presented by other donors, and the most interesting of the books purchased during 1979 are listed at the end of this Bulletin.

Previous Bulletins have presented short biobibliographies of Willan, Bateman, A.T. Thomson and Thomas Hillier—important figures in the main stream of dermatology in Britain. In contrast this issue contains a biobibliography of A.B. Squire, who was never a member of either of the dermatological societies which played so large a part in developing the speciality in Britain, but whose numerous publications made important contributions by pioneering the use of photography in teaching and by careful investigations of the value of chrysarobin in psoriasis.

The Willan Librarian will be glad to hear from readers who have old or rare works on dermatology, which they are willing to offer to the library. The Library is housed in the Willan Room of the Royal College of Physicians, London. Historians of dermatology wishing to visit the library should apply to the Willan Librarian.

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Honorary Willan Librarian

The four previous Willan Library Bulletins, written by Dr Rook, were produced as leaflets and distributed with the Journal.

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## ALEXANDER BALMANNO SQUIRE 1836–1908

Alexander John Balmanno Squire was born in London in 1836, the second son of Peter Squire, chemist to Queen Victoria. His younger brothers Peter Wyatt Squire and Alexander Herbert Squire succeeded to their father's pharmaceutical business at 413 Oxford Street. Alexander's father, a pharmacist of great distinction, was appointed chemist to the Princess Victoria in 1836, and in the following year was officially gazetted chemist to the Queen. The Squire family had long farmed in the St Neots area of Huntingdonshire, and some of them still do so. Alexander's mother was the daughter of Alexander Balmanno, a prosperous London merchant.

Alexander's choice of University College as a medical school may well have been influenced by the fact that his cousin William Squire (1825–1899), later a well known physician and epidemiologist, had studied there. He may also have been influenced by Sir William Jenner, who as physician to the Queen from 1861 was well known to Peter Squire, her chemist. It is certain that Jenner gave Alexander his active support and launched him on his career as a dermatologist.

Alexander qualified M.R.C.S. L.S.A. from University College Hospital in 1858. In 1862 he was M.B. London, winning a gold medal. From 1853 until 1861 Jenner was in charge of skin patients at the hospital to which he was of course also physician. Alexander was Jenner's pupil as a student. He later served as House Surgeon to the hospital, and as Medical Officer to the Marylebone General Dispensary. This was a long established charity in Welbeck Street; the senior physician C.J. Hare was physician to University College Hospital and, from 1863, professor of clinical medicine there. Squire then joined the staff of the West London Free Dispensary for Diseases of the Skin, 45 Dorset Street, Baker Street. This institution, which has understandably been confused with the Western Dispensary for Diseases of the Skin, was founded in 1843, and during its short life attracted staff of high repute. Squire's predecessor as surgeon was J.E. Erichsen, later to become professor of Surgery at University College Hospital. The Western Dispensary, founded in 1851, survived for almost a century and was associated for its first three decades with T. Hunt the advocate and popularizer of arsenic for almost everything. Squire resigned from the West London Free Dispensary in 1864 to become Surgeon to the British Hospital for Diseases of the Skin, which he had founded in Great Marlborough Street. Later, branches were opened in Newington Butts and in Finsbury Square. Sir William Jenner, influential and greatly respected, agreed to be named as consulting physician to the new hospital and by so doing probably protected the hospital to some extent from the virulent attacks by the medical press which were directed at many of the other special hospitals. However, Squire and his hospital did not entirely escape hostile criticism. Squire was a pioneer in the use of photography in dermatological illustration. In 1870 'the large theatre of the Polytechnic Institute was the scene of a very curious gathering and a still more curious entertainment'(1). Squire had invited members of the profession 'to witness an attempt to demonstrate diseases of the skin by means of the dissolving view apparatus'. About 2000 people were present. The writer of the editorial praised the photographs and the projectionist but thought that there was 'not a shadow of a need for such an addition to the existing resources of lecturers'. He considered that from 'a professional point of view the scene was one of a profoundly depressing character'. He went on to condemn the British Hospital for taking patients and therefore money away from general practitioners. The hospital continued to prosper and during 1890 3472 new patients attended (2). In 1907, as his health was failing, Squire decided to give up his hospital work. The hospital committee placed its funds in the hands of the Charity Commissioners to found and endow charities for the benefit of patients with skin disease at the Charing Cross Hospital, Guy's Hospital and the Westminster Hospital (3), Squire died at his home at 24 Weymouth Street on 11 May 1908.

Squire was never a member of the Dermatological Society of London or of the more eclectic

Dermatological Society of Great Britain and Ireland (4), although he was a member and frequent speaker at the Royal Medico-Chirurgical Society (predecessor of the Royal Society of Medicine), the Medical Society of London, the Clinical Society and the Section of Dermatology at the Annual Meetings of the British Medical Association. His very numerous publications are conveniently classified in four categories: photographic atlases, general texts, papers on psoriasis and its treatment, miscellaneous contributions.

### *Atlases*

Squire, A.B. (1865) *Photographs (Coloured from Life) of the Diseases of the Skin.* Churchill, London. (W.L. incomplete set.)

Squire, A.B. (1866) *Chromolithographs (after Coloured Photographs from Life) of the Diseases of the Skin.* Churchill, London. (W.L. incomplete set.)

These photographs, which were the first to be published of skin diseases in England, were very favourably received by the reviewers. 'We thank him for what he has already done and are glad to hear that he has announced his intention of bringing out another series of a similar kind' (5).

Squire, A.B. (1878–1889) *Atlas of Diseases of the Skin* (published in parts at irregular intervals). Churchill, London. (W.L. incomplete set.)

### *General texts*

Squire, A.B. (1868) *A Manual of Diseases of the Skin,* p. 276. Churchill, London. (1869) Second Edition. (1885) Third Edition. (1887) Fourth Edition.

Squire, A.B. (1867) *Unhealthy Skin: Its Prevention and Management. A Popular Treatise on Cutaneous Hygiene,* p.p. 148. Longman, Green & Co., London. (W.L.)

Reynolds, J.R. (1866–1879) *A System of Medicine.* Five volumes. Articles on Dermatology by A.B. Squire.

Squire, A.B. (1889) *Pharmacopoeia of the British Hospital for Diseases of the Skin.* Churchill, London.

Squire's 'Manual' enjoyed a modest success. It shows no great originality, but is sound and critical on treatment, and warns about the disadvantages of arsenic, the carcinogenic properties of which were first pointed out by Jonathan Hutchinson some years later. In his book written for the layman he was following in the tradition set by Erasmus Wilson. Like Wilson and other later dermatologists who have attempted this difficult task he was accused by some of advertising.

Sir James Russell Reynolds (1828–1896) trained at University College Hospital to which he was elected assistant physician in 1859. He succeeded Jenner as Professor of Medicine in 1867. His 'System' was a standard work and the inclusion of Squire amongst the authors suggests that he enjoyed the respect of the medical establishment. Reynolds was P.R.C.P. 1893–6.

The Pharmacopoeia of the British Hospital, edited by Squire, reached a third edition in 1884.

### *Writings on psoriasis*

Squire introduced the treatment of psoriasis with chrysarobin. The use of Goa Powder as an Indian treatment for ringworm was advocated by Sir John Fayrer in 1874. Squire in 1876 (6) reported that he had seen a patient with annular psoriasis misdiagnosed as ringworm, and successfully treated with Goa Powder. Professor Attfield (7) had analysed the powder and found it to consist of 85% chrysophanic acid; he pointed out that 'Goa' powder in fact originated in Brazil. Squire had successfully treated two cases of psoriasis with chrysophanic acid ointment. Attfield's analysis was subsequently shown to be incorrect, but the treatment of psoriasis with chrysarobin gradually became established.

Recent work on the effect of simple occlusion on the lesions of psoriasis gives added interest to

Squire's report of two patients whose psoriasis cleared after they had worn india-rubber underclothing for two weeks (8). He later reported that these patients had developed 'some slight' recurrence, but that the treatment 'always does some tangible good'(9).

Sidney Ringer, a distinguished pharmacologist, carried out a prospective trial of tar taken orally for the treatment of chronic bronchitis(10). In 1875 the concept of controlled trials was unfamiliar even to a scientist of Ringer's calibre. He administered tar to twenty-five out-patients and assessed their response subjectively. He reported that tar 'is a good, useful though perhaps not a striking remedy'. Squire (11) wrote to report internal tar as of no value in psoriasis. M'Call Anderson (12) of Glasgow promptly wrote to express his strongly held opinion that internal tar was one of the most valuable treatments for psoriasis! Squire too, did not use controls, but he was critical in his assessment of therapeutic claims. He failed to confirm Kaposi's claim that 10% $\beta$ naphthol was effective in psoriasis (13).

### *Miscellaneous contributions*

For over three decades Squire published a succession of papers and case reports on a wide variety of dermatological topics. He was quick to adopt new methods of treatment. He modified Vidal's linear scarificator, which he used in the treatment of lupus (14) and port wine naevi (15). One of his last publications was on the use of electrolysis in hirsutism (16).

### *Conclusions*

Alexander Balmanno Squire was a dermatologist of wide experience with a special interest in therapeutics. His most important contribution was the introduction of chrysarobin for the treatment of psoriasis. One can only speculate as to what factors denied him recognition by contemporary dermatologists in Britain, even to the limited degree indicated by election to the far from exclusive Dermatological Society of Great Britain and Ireland. His contemporaries in general medicine and surgery thought well of him and the German Dermatological Society elected him a member.

## ACKNOWLEDGMENT

Mr R.G.Todd, Librarian of the Pharmaceutical Society of Great Britain, kindly provided information about the Squire family.

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14. Squire, B. (1880) On the treatment of lupus by linear scarification. *British Medical Journal*, **i**, 654.
15. Squire, B. (1879) An improvement in the treatment of port wine mark by linear scarification. *British Medical Journal*, **ii**, 732.
16. Squire, B. (1905) Electrolysis: an improved method of operating on superfluous hair. *Lancet*, **i**, 493.

## ACCESSIONS TO THE WILLAN LIBRARY 1979

Accessions during the year included:

H.E.Cauty (1874) *Diseases of the Skin in Twenty-four Lectures on the Principles and Practice of Cutaneous Medicine.* Pp. x + 365. Churchill, London (purchased).

Henry Evans Cauty was surgeon to the Liverpool Dispensary for Diseases of the Skin. He dedicates the volume to Edward Balman; 'the first provincial institution for the study and relief of cutaneous diseases owes its foundation to your brother, the late Dr Balman and yourself'.

T.M'Call Anderson (1887) *A Treatise on Diseases of the Skin with Special Reference to their Diagnosis and Treatment*, including an analysis of 11,000 consecutive cases. Pp. xviii + 639. Charles Griffin, London (purchased).

Sir Thomas M'Call Anderson (1836–1908) of Glasgow was an influential and respected figure in British medicine in the last generation of the nineteenth century. In 1861 he founded the Glasgow Hospital for Diseases of the Skin. He was Professor of Medicine at Anderson College, the Professor of Clinical Medicine, and, in 1900, Regius Professor of Medicine of the University of Glasgow. His textbook, of which this is the first edition, was an important work and was the leading British text until the publication of Radcliffe Crocker's text.

Sten Hörstadius (1950) *The Neural Crest.* Pp. ix + 111. Oxford University Press, Oxford. (reprint 1969) by Hafner Publishing Company) (presented by Dr and Mrs Robert Jackson in memory of Dr Ian Whimster).

H.D.Speransky (1943) *A Basis for the Theory of Medicine.* Translated and edited by C.P.Dutt. Pp. 417. N.Y. International Publications (presented by Dr and Mrs Robert Jackson in memory of Dr Ian Whimster).

Arne Kissmeyer (1928) *Anne-Charles Lorry* (1726–1783) *et son Oeuvre Dermatologique.* Préface du Professeur Louis Brocq. Pp. xv + 64. Librarie Amédie Legrand, Paris (purchased).

In his preface Brocq refers to Lorry as 'the true founder of dermatological science', but admits that he has never read his works as the latin text had not been translated.

R.Cranston Low (1911) *Carbonic Acid Snow as a Therapeutic Agent in the Treatment of Diseases of the Skin.* Pp. xi + 117. Green, London (purchased).

J.Hall-Edwards (1913) *Carbon Dioxide Snow: its Therapeutic Uses.* Pp. xv + 78. Simpkin Marshall, London (purchased).

Treatment of skin diseases by refrigeration was introduced in 1899 by Campbell White, who used liquid air. Juliusberg of Breslau in 1905 used carbonic acid as a spray; Pusey of Chicago, in the same year used carbonic acid in its solid form.

Cranston Low, a well known Edinburgh dermatologist, had worked in Breslau. Hall-Edwards was in charge of the X-ray department at the General Hospital, Birmingham.

F.Parkes Weber's Collected Writings. A classified and indexed bibliography edited by the Medical Staff of the German Hospital, London, in celebration of his 80th birthday, May 8th 1943, and the 50th Anniversary of his appointment of Physician to the hospital (purchased).

Francesco Radaeli (1934) *Malattie Cutanée*, 4th edition. Pp. xv + 776. Vallardi, Milan (purchased).

Erasmus Wilson (1853) *Hufeland's Art of Prolonging Life* (edited by Erasmus Wilson). Pp. xvi + 271. Churchill, London (purchased).

Christian Wilhelm Hufeland (1762–1836) was Professor of Medicine first at Jena and later at Berlin. He was the friend and physician of Goethe, Schiller and Herder. He was a great pioneer of medical journalism and also published several works on popular medicine, of which the best known was *The Art of Prolonging Life* (1796). The American medical historian Fielding Garrison, maintained that Hufeland 'was one of the great philanthropic physicians who are true friends of the human race'.

An English translation of *The Art of Prolonging Life* was published in 1797. Erasmus Wilson used this version, but 'to adapt the work to the modes of thinking and feeling of the present day, certain truths, too true to be allowed to stand forth in all their naked proportions, required a veil to be thrown around them'.

M.A.Cullerier (1866) *Précis Iconographique des Maladies Vénériennes*. Pp. 488. Masson, Paris (presented by Dr Stig Hard).

The seventy-four plates, steel engravings by Léveille, provide illustrations of very high quality for the period.

Karl Kopp (1893) *Atlas der Hautkrankheiten*. 90 col. plates, 17 B/w. Lehmann, Munich (presented by Dr Stig Hård).

Raymond Barthélemy (1921) *Dermatologie Usuelle*. Pp. 138. Arnette, Paris (presented by Dr Stig Hard).

Balzer in his preface praises the work as a practical vade mecum by a young dermatologist.

Dr Stig Hård has presented also the following interesting offprints:

Schaumann, J. (1917) Recherches sur le lupus pernio et ses Relations avec les Sarcoïdes cutanées et sous-cutanées. *Nordiskt Medicinskt Arkiv*, **6**, 1–81.

Schaumann, J. (1936) Lymphogranulomatosis benigna in the Light of Prolonged Clinical Observation and Autopsy Findings. *British Journal of Dermatology*, **48**, 399–446.

Jörgen Schaumann began his classic studies of sarcoidosis at St Goran's Hospital, Stockholm, and continued them at the Finsen Institute of which he became Director.

Welander, E. (1895) Fehlerhefte Zahnbildung bei erworbener Syphilis. *Nordiskt Medicinskt Arkiv*, **16**, 1–18.

A clear account of the dental changes in congenital syphilis.

Physicians and Surgeons of the Principal London Hospitals (1884) *The Family Physician; a Manual of Domestic Medicine*. Four volumes. Cassell, London (presented by Dr Ian Martin Scott).

The authors of this large work made their contributions anonymously, in order to avoid any suspicion of advertising. Skin diseases occupy a small section of their own in Volume III, but others are discussed in other volumes.

A.Clarke (1820) *An Essay on Warm Cold and Vapour Bathing*. 5th Edition, Pp. xii–232. Colburn, London (purchased).

Jonathan Green (1823) *The Utility and Importance of Fumigating Baths*. Pp. 115. Adlard, London (purchased).

Baths were an important therapeutic development in the nineteenth century. Warm or cold baths were advocated for many skin diseases but medicated baths were used to good effect in scabies and 'fumigations' were given in scabies and in syphilis, amongst other conditions.

Sir Arthur Clarke was a general practitioner in Dublin. Jonathan Green (1788–1864) served in the Navy, then practised for many years as a dermatologist in the West End of London. He was the author of a small textbook, which enjoyed some success and was translated into German.

Sir Philip Manson-Bahr (1956) *History of the School of Tropical Medicine in London* (1899–1949). Pp. xiii–329. Lewis, London (purchased).

This standard history of the School includes biographical notes on the members of the staff. Sir James Galloway was the first dermatologist; he joined the staff in 1900. He resigned in 1902, and was succeeded by J.M.H.Macleod.

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| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$ g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$ g) | $\mu$g | cubic millimetre(s) | mm$^3$ | micrometre(s) | $\mu$m |
| nanogram(s) ($10^{-9}$ g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$ g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hour(s) | h | milliequivalent | mmol | | |

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